

Supporting Information

Coibamide A Targets Sec61 to Prevent Biogenesis of Secretory and Membrane Proteins

Dale Tranter,¹⁺ Anja O. Paatero,¹⁺ Shinsaku Kawaguchi,² Soheila Kazemi,³ Jeffrey D. Serrill,³ Juho Kellosalo,¹ Walter K. Vogel,³ Uwe Richter,⁴ Daphne R. Mattos,³ Xuemei Wan,³ Christopher C. Thornburg,⁵ Shinya Oishi,² Kerry L. McPhail,³ Jane E. Ishmael³ and Ville O. Paavilainen^{1*}

¹Institute of Biotechnology, University of Helsinki, Helsinki, 00014, Finland

²Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

³Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, USA

⁴Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki

⁵Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland 21702

⁺These authors contributed equally

*Corresponding author

Correspondence:

Ville Paavilainen, Ph.D.
Institute of Biotechnology, University of Helsinki
Viikinkaari 1, Biocenter 3
00014 Helsinki, Finland

Phone: +358-50-448 4600

E-mail: ville.paavilainen@helsinki.fi

Table of Contents

COIBAMIDE COMPOUND SYNTHESES	S4
BIOLOGICAL TESTING OF SYNTHETIC COIBAMIDE ANALOGUES FOR PROBE DEVELOPMENT	S11
HPLC CHROMATOGRAMS OF PURIFIED CBA DERIVATIVES.....	S12
¹ H NMR SPECTRA FOR CBA DERIVATIVES	S16
US NATIONAL CANCER INSTITUTE 60 CANCER CELL LINE (NCI60) PANEL MATRIX COMPARE ANALYSIS.	S34
COMPARISON OF PUBLISHED NCI60 PANEL DATA FOR COIBAMIDE A, APRATOXIN A AND IPOMOEASSIN F	S35
REFERENCES	S35

Coibamide Compound Syntheses

General Experimental.

¹H NMR spectra were recorded using a JEOL ECA-500 spectrometer. Chemical shifts are reported in δ (ppm) relative to Me₄Si (in CDCl₃) as an internal standard. ¹³C NMR spectra were recorded using a JEOL ECA-500 spectrometer and referenced to the residual solvent signal. Exact mass (HRMS) spectra were recorded on a Shimadzu LC-ESI-IT-TOF-MS equipment. Optical rotations were measured with a JASCO P-1020 polarimeter. For flash chromatography, Wakogel C-200E (Wako) was employed. For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 \times 250 mm, Nacalai Tesque, Inc.) was employed with a linear gradient of CH₃CN (with 0.1% (v/v) TFA) in H₂O at a flow rate of 1 mL/min, and eluting products were detected by UV at 220 nm. Preparative HPLC was performed using a Cosmosil 5C18-ARII preparative column (20 \times 250 mm, Nacalai Tesque, Inc.) at a flow rate of 8 mL/min.

2-Oxo-2-phenylethyl (*R*)-2-hydroxy-3-methylbutanoate (S2). To a stirred solution of (*R*)-2-hydroxy-3-methylbutyric acid **S1** in THF (70.0 mL) were added phenacyl bromide (4.97 g, 30.0 mmol), and Et₃N (3.16 mL, 22.7 mmol) at room temperature. After being stirred for 1h, the mixture was quenched with aqueous saturated NH₄Cl. The whole was extracted with EtOAc and the extract was washed with brine and dried over MgSO₄. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexanes–EtOAc (2:1) to give compound **S2** (4.19 g, 78%) as a colorless oil: [α]²⁷_D –11.9 (c 0.50, CHCl₃); HRMS (FAB) calcd for C₁₃H₁₇O₄ [M+H]⁺: 237.1127; found: 237.1123.

Fmoc-Val-D-Hiva-OPac (S3). To a stirred solution of alcohol **S2** in THF (35.0 mL) were added DCC (7.30 g, 35.4 mmol), DMAP (216 mg, 1.77 mmol) and Fmoc-L-Val-OH (7.23 g, 21.3 mmol) at room temperature. After being stirred overnight, the mixture was quenched with aqueous saturated NH₄Cl. The whole was extracted with EtOAc and the extract was washed with brine and dried over MgSO₄. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography over silica gel with hexanes–EtOAc (2:1) to give compound **S3** (6.22 g, 63%) as colorless crystals: [α]²⁷_D –12.9 (c 0.15, CH₃CN); HRMS (ESI-TOF) calcd for C₃₃H₃₆NO₇ [M+H]⁺: 558.2492; found: 558.2467.

Fmoc-Val-D-Hiva-OH (S4). To a stirred solution of alcohol **S3** in AcOH/EtOAc/H₂O (60:35:5, 224 mL) were added Zn (10.9 g, 167 mmol) at room temperature. After being stirred for 24h, the mixture was filtered through Celite, and 1M HCl was added to the filtrate. The whole was extracted

with EtOAc and the extract was washed with brine, and dried over MgSO₄. The filtrate was concentrated under reduced pressure and AcOH was removed by azotropic distillation with toluene. The residue was purified by flash chromatography over silica gel with hexanes–EtOAc (1:1) to give compound **S2** (4.42 g, 90%) as a colorless solid: [α]²⁷_D –12.7 (*c* 0.39, CH₃CN); HRMS (ESI-TOF) calcd for C₂₅H₃₀NO₆ [M+H]⁺: 440.2073; found: 440.2072.

Solid-Phase Peptide Synthesis.

Loading of the C-terminal amino acid on (2-Cl)Trt resin. To a solution of the Fmoc-Tyr(Me)-OH (806 mg) in CH₂Cl₂ (21.4 mL) was added (i-Pr)₂NEt (1.36 mL, 7.80 mmol). The whole was poured into Cl-(2-Cl)Trt resin (1.52 mmol/g, 2.14 g, 3.25 mmol), and the reaction was continued for 2 h to give the resin.

Deprotection of Fmoc group. The Fmoc-protected peptidyl resin was treated with piperidine/DMF (2:8) for 20 min.

Coupling reaction using DIC-HOAt [for MeSer(Me)3, MeSer(Me)6, MeThr5 and Ala8]. N,N'-Diisopropylcarbodiimide (DIC, 311 μL, 2.00 mmol) was added to a solution of Fmoc-Ser(Me)-OH (682 mg, 2.00 mmol) and HOAt (274 mg, 2.00 mmol) in DMF (7.43 mL). The whole was poured into the peptidyl resin, and the reaction was continued for 2 h.

Coupling reaction using DIC-HOBt [for MeLeu9]. DIC (882 μL, 5.70 mmol) was added to a solution of Fmoc-MeLeu-OH (2.00 g, 5.70 mmol) and HOBt·H₂O (873 mg, 5.70 mmol) in DMF (21.7 mL). The whole was poured into the peptidyl resin, and the reaction was continued for 2 h.

Coupling reaction using HATU-(i-Pr)₂NEt [for Fmoc-Val1-D-Hiva2]. To a solution of Fmoc-Val-D-Hiva-OH (2.57 g, 5.85 mmol) in DMF (21.4 mL) were added HATU (2.15 g, 5.66 mmol) and (i-Pr)₂NEt (2.03 mL, 11.7 mmol). The whole was poured into the peptidyl resin, and the reaction was continued for 2 h.

On-resin N-methyl modification of α-amino group for MeSer(Me)6. The peptide resin (0.20 mmol) was treated with o-nitrobenzenesulfonyl chloride (221 mg, 1.00 mmol) and 2,4,6-collidine (246 μL, 1.00 mmol) in NMP for 30 min at room temperature. To the N-Ns-protected resin in anhydrous THF were added MeOH (81.0 μL, 2.00 mmol), PPh₃ (262 mg, 1.00 mmol), and diethyl diazodicarboxylate (455 μL, 1.00 mmol) at room temperature. The suspension was shaken for 30 min (x2) at room temperature. The N-methylated resin was treated with DBU (150 μL, 2.00 mmol) and 2-mercaptoethanol (140 μL, 1.00 mmol) for 5 min at room temperature to give the N-methylated peptide resin.

Coupling of fragment 1 with fragment 2. DIC (15.5 μL, 0.10 mmol) was added to a solution of peptide **S7** (13.6 mg, 0.10 mmol), HOAt (13.6 mg, 0.10 mmol) and (i-Pr)₂NEt (34.8 μL, 0.20 mmol)

in DMF (372 μ L). The whole was poured into the peptidyl resin **S8**, and the reaction was continued for 6 h.

Coupling of D-MeAla10 with MeThr5 hydroxy group. Fmoc-D-Ala-OH (97.6 mg, 0.30 mmol), DIC (46.4 μ L, 0.30 mmol) and DMAP (11.0 mg, 0.09 mmol) were added to the resin **S9** in DCE. The reaction was continued for 3 h.

Coupling of fragment 3 with D-MeAla11. To a solution of fragment 3 (**S13**) in DMF (372 μ L) were added DIC (28.0 μ L, 0.18 mmol) and HOAt (24.5 mg, 0.18 mmol). The whole was poured into the resin **S10**, and the reaction was continued for 3 h.

Cleavage from the resin. The peptidyl resin was treated with 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP)/CH₂Cl₂ (3:7) at room temperature for 2 h. After removal of the resin by filtration, the filtrate was concentrated under reduced pressure to give crude peptide. Fragment 1 and the derivatives were purified by preparative HPLC. Fragment 3 and the derivatives were purified by column chromatography over silica gel.

Me₂Val-D-Hiva-MeSer(Me)-MeLeu-OH (S7). 426 mg, 72% from resin, colorless solid: $[\alpha]^{27}_D$ – 40.0 (c 0.80, CHCl₃); HRMS (ESI-TOF) calcd for C₂₄H₄₆N₃O₇ [M+H]⁺: 488.3330; found: 488.3334.

Me₂Val-D-Hiva-MePra-MeLeu-OH ([MePra3]-S7). 332 mg, 35% from resin, colorless solid: $[\alpha]^{27}_D$ – 57.1 (c 0.83, CHCl₃); HRMS (ESI-TOF) calcd for C₂₅H₄₄N₃O₆ [M+H]⁺: 482.3224; found: 482.3219.

Me₂Val-D-Hiva-MeSer(Me)-MePra-OH ([MePra4]-S7). 353 mg, 38% from resin, colorless solid: $[\alpha]^{27}_D$ – 44.3 (c 0.99, CHCl₃); HRMS (ESI-TOF) calcd for C₂₃H₄₀N₃O₇ [M+H]⁺: 470.2860; found: 470.2858.

Fmoc-Ala-MeLeu-Tyr(Me)-OH (S13). 1.43 g, 92% from resin, colorless solid: $[\alpha]^{27}_D$ – 48.0 (c 0.84, CHCl₃); HRMS (ESI-TOF) calcd for C₃₅H₄₁N₃NaO₇ [M+Na]⁺: 683.2836; found: 683.2840.

Fmoc-Pra-MeLeu-Tyr(Me)-OH ([Pra8]-S13). 1.11 g, 91% from resin, colorless solid: $[\alpha]^{27}_D$ – 44.9 (c 0.74, CHCl₃); HRMS (ESI-TOF) calcd for C₃₇H₄₁N₃NaO₇ [M+Na]⁺: 662.2836; found: 662.2838.

Fmoc-Ala-MePra-Tyr(Me)-OH ([MePra9]-S13). 1.05 g, 96% from resin, colorless solid: $[\alpha]^{27}_D$ – 70.4 (c 1.25, CHCl₃); HRMS (ESI-TOF) calcd for C₃₄H₃₅N₃NaO₇ [M+Na]⁺: 598.2553; found: 598.2555.

Fmoc-Ala-MeLeu-Tdf-OH ([Tdf10]-S13). 355 mg, 91% from resin, colorless solid: $[\alpha]^{27}_D -48.6$ (*c* 0.57, CHCl₃); HRMS (ESI-TOF) calcd for C₃₆H₃₈F₃N₅NaO₆ [M+Na]⁺: 716.2666; found: 716.2667.

Peptide S11. 65.5 mg, 20% from resin, colorless solid: $[\alpha]^{27}_D -140.6$ (*c* 0.23, CHCl₃); HRMS (ESI-TOF) calcd for C₆₅H₁₁₄N₁₀O₁₇ [M+2H]²⁺: 635.4176; found: 635.4178.

Peptide [MePra3]-S11. 23.0 mg, 15% from resin, colorless solid: $[\alpha]^{27}_D -125.0$ (*c* 0.14, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₁₂N₁₀O₁₆ [M+2H]²⁺: 650.4123; found: 650.4124.

Peptide [MePra4]-S11. 36.8 mg, 13% from resin, colorless solid: $[\alpha]^{27}_D -133.5$ (*c* 0.35, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₈N₁₀O₁₇ [M+2H]²⁺: 644.3941; found: 644.3940.

Peptide [MePra6]-S11. 49.2 mg, 17% from resin, colorless solid: $[\alpha]^{27}_D -135.4$ (*c* 0.40, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₁₂N₁₀O₁₆ [M+2H]²⁺: 650.4123; found: 650.4123.

Peptide [MePra7]-S11. 109 mg, 12% from resin, colorless solid: $[\alpha]^{27}_D -117.1$ (*c* 0.38, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₈N₁₀O₁₇ [M+2H]²⁺: 644.3941; found: 644.3937.

Peptide [Pra8]-S11. 165 mg, 23% from resin, colorless solid: $[\alpha]^{27}_D -115.3$ (*c* 0.26, CHCl₃); HRMS (ESI-TOF) calcd for C₆₇H₁₁₄N₁₀O₁₇ [M+2H]²⁺: 665.4176; found: 665.4174.

Peptide [MePra9]-S11. 16.6 mg, 21% from resin, colorless solid: $[\alpha]^{27}_D -107.1$ (*c* 0.18, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₈N₁₀O₁₇ [M+2H]²⁺: 644.3941; found: 644.3939.

Peptide [Tdf10]-S11. 17.7 mg, 32% from resin, colorless solid: $[\alpha]^{27}_D -74.3$ (*c* 0.17, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₁₁F₃N₁₂O₁₆ [M+2H]²⁺: 692.4091; found: 692.4092.

Peptide [MePra3,Tdf10]-S11. 13.0 mg, 29% from resin, colorless solid: $[\alpha]^{27}_D -86.8$ (*c* 0.37, CHCl₃); HRMS (ESI-TOF) calcd for C₆₇H₁₀₉F₃N₁₂O₁₅ [M+2H]²⁺: 689.4038; found: 689.4034.

Macrocyclization of Open-Chain Peptide: CbA (S12). To a stirred solution of peptide **S11** (32.0mg, 0.0245 mmol) in CH₂Cl₂ (80.0 mL) were added HOAt (33.3 mg, 0.245 mmol), EDCI·HCl (47.0 mg, 0.245 mmol) and (i-Pr)₂NEt (170 μ L, 0.98 mmol) at 0 °C. After being stirred for 8 h at room temperature, the reaction mixture was concentrated under reduced pressure, and CH₂Cl₂

and aqueous saturated NH₄Cl were added to the residue. The whole was extracted with CH₂Cl₂ and the extract was washed with brine, aqueous saturated NaHCO₃ and brine, dried over MgSO₄. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase preparative HPLC (62% CH₃CN in H₂O) to give CbA (**S12**) (13.0 mg, 41%) as a colorless powder. The spectral data were in good agreement with those previously reported. HRMS (ESI-TOF) calcd for C₆₅H₁₁₀N₁₀NaO₁₆ [M+Na]⁺: 1309.7993; found: 1309.7994.

Peptide [MePra3]-S12. 3.8 mg, 18%, colorless powder: $[\alpha]^{27}_D -106.4$ (c 0.15, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₀₉N₁₀O₁₅ [M+H]⁺: 1281.8068; found: 1281.8067.

Peptide [MePra4]-S12. 15.1 mg, 23%, colorless powder: $[\alpha]^{27}_D -130.4$ (c 0.71, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₅N₁₀O₁₆ [M+H]⁺: 1269.7704; found: 1269.7701.

Peptide [MePra6]-S12. 6.8 mg, 16%, colorless powder: $[\alpha]^{27}_D -126.0$ (c 0.34, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₀₉N₁₀O₁₆ [M+H]⁺: 1281.8068; found: 1281.8070.

Peptide [MePra7]-S12. 1.7 mg, 28%, colorless powder: $[\alpha]^{27}_D -114.6$ (c 0.17, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₅N₁₀O₁₆ [M+H]⁺: 1269.7704; found: 1269.7709.

Peptide [Pra8]-S12. 13.2 mg, 18%, colorless powder: $[\alpha]^{27}_D -152.4$ (c 0.61, CHCl₃); HRMS (ESI-TOF) calcd for C₆₇H₁₁₁N₁₀O₁₆ [M+H]⁺: 1311.8174; found: 1311.8177.

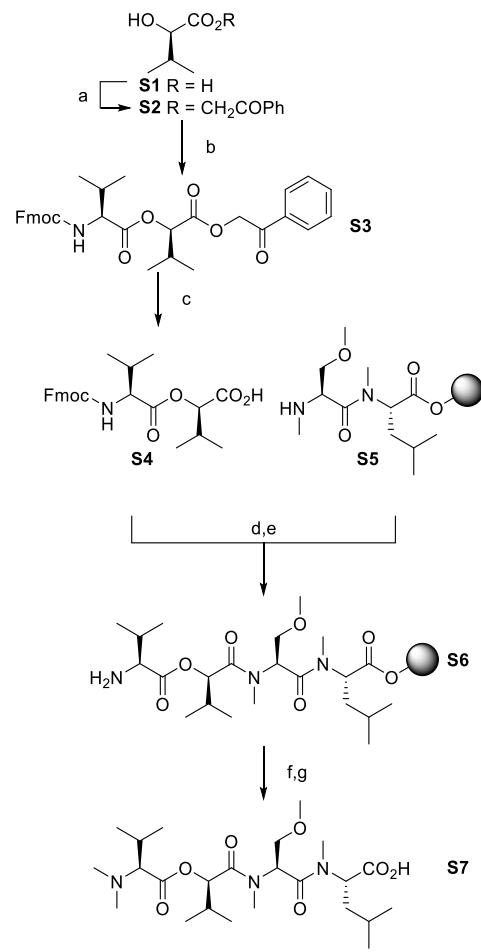
Peptide [MePra9]-S12. 5.4 mg, 21%, colorless powder: $[\alpha]^{27}_D -122.5$ (c 0.25, CHCl₃); HRMS (ESI-TOF) calcd for C₆₄H₁₀₅N₁₀O₁₆ [M+H]⁺: 1269.7704; found: 1269.7701.

Peptide [Tdf10]-S12. 5.1 mg, 29%, colorless powder: $[\alpha]^{27}_D -142.9$ (c 0.25, CHCl₃); HRMS (ESI-TOF) calcd for C₆₆H₁₀₈F₃N₁₂O₁₅ [M+H]⁺: 1365.8003; found: 1365.8007.

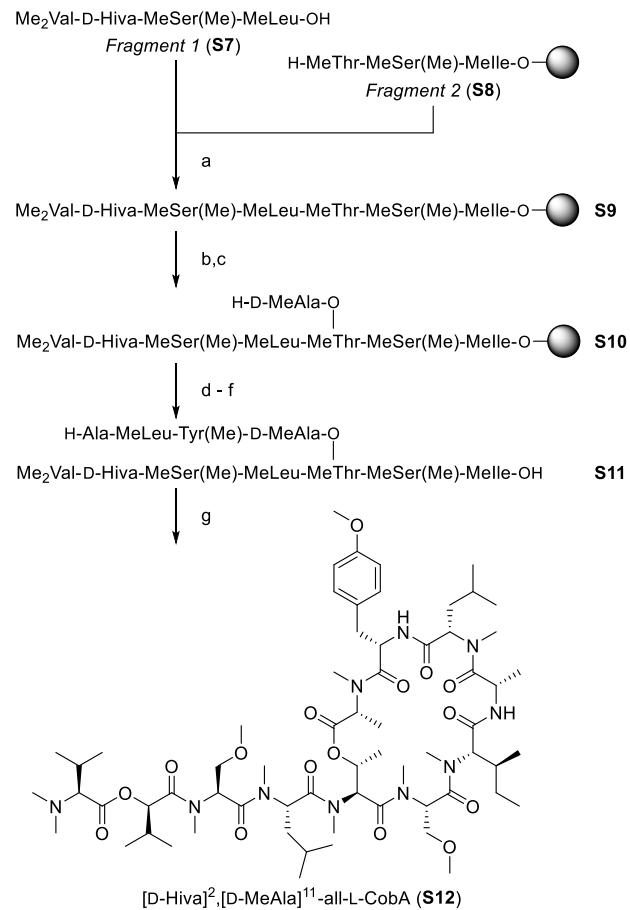
Peptide [MePra3,Tdf10]-S12. 3.0 mg, 49%, colorless powder: $[\alpha]^{27}_D -154.2$ (c 0.15, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 0.62-1.20 (m, 26H), 1.21-1.63 (m, 15H), 1.71 (m, 1H), 1.78-2.15 (m, 6H), 2.31 (m, 2H), 2.43 (s, 3H), 2.50-3.25 (m, 27H), 3.30 (s, 3H), 3.51 (m, 1H), 3.69 (m, 3H), 3.88 (m, 1H), 4.73 (t, J = 6.6 Hz, 1H), 5.16 (m, 3H), 5.35 (m, 1H), 5.50 (m, 1H), 5.72 (m, 1H), 6.06 (m, 1H), 6.33 (br s, 1H), 6.57 (br s, 1H), 6.78 (br s, 1H), 7.13 (m, 2H), 7.26 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 11.6, 12.7, 13.0, 15.8, 16.1, 17.6, 18.4, 18.5, 18.7, 19.2, 20.0, 20.6, 21.2, 23.2,

23.3, 23.6, 24.3, 25.2, 28.3, 28.7, 28.9, 29.5, 29.7, 29.8, 30.3, 31.1, 32.0, 36.4, 37.3, 37.6, 39.0, 44.6, 47.0, 51.1, 51.6, 52.8, 53.0, 53.7, 58.8, 64.7, 68.5, 70.4, 71.5, 80.1, 126.5, 130.0, 167.2, 167.7, 168.3, 168.5, 169.9, 171.0, 171.1; HRMS (ESI) calcd for $C_{67}H_{105}F_3N_{12}O_{14}$ [M + H] $^+$: 1359.7898; found: 1359.7893.

Scheme S1. Synthesis of Fragment 1 and the Derivatives. Reagents and conditions: (a) phenacyl bromide, (*i*-Pr)₂NEt, (b) Fmoc-L-Val-OH, EDCI-HCl, DMAP, CH₂Cl₂, (c) Zn, AcOH, H₂O, EtOAc, (d) HATU, (*i*-Pr)₂NEt, DMF, (e) 20% piperidine / DMF, (f) 30% HFIP / CH₂Cl₂, (g) NaBH(OAc)₃, AcOH, CH₂Cl₂.



Scheme S2. Solid-phase synthesis of CbA and the analogues. Reagents and conditions: (a) DIC, HOAt, (*i*-Pr)₂NEt, DMF, (b) Fmoc-D-MeAla-OH, DIC, DMAP, (c) 20% piperidine / DMF, (d) Fmoc-Ala-MeLeu-Tyr(Me)-OH (*Fragment 3, S13*), DIC, HOAt, DMF, (e) 20% piperidine / DMF. (f) 30% HFIP / CH₂Cl₂. (g) EDCI·HCl, HOAt, (*i*-Pr)₂NEt, CH₂Cl₂.



Biological Testing of Synthetic Coibamide Analogues for Probe Development

Growth inhibition assays using A549 human lung adenocarcinoma cells were performed in 96-well plates (BD Falcon) as described previously. A549 cells were seeded at 500 cells/well in 50 μL of culture media, respectively, and were cultured for 6 h. Chemical compounds in DMSO were diluted 250-fold with the culture medium in advance. Following the addition of 40 μL of the fresh culture medium to the cell cultures, 30 μL of the chemical diluents were also added. The final volume of DMSO in the medium was equal to 0.1% (v/v). The cells under chemical treatment were incubated for further 72 h. The wells in the plates were washed twice with the cultured medium without phenol-red. After 1-hour incubation with 100 μL of the medium, the cell culture in each well was supplemented with 20 μL of the MTS reagent (Promega), followed by incubation for additional 40 min. Absorbance at 490 nm of each well was measured using a Wallac 1420 ARVO SX multilabel counter (Perkin Elmer). Three experiments were performed per condition and the averages of inhibition rates in each condition were evaluated to determine IC₅₀ values using GraphPad Prism software.

Peptide	IC ₅₀ (nM)
coibamide A (CbA)	2.1 ± 0.7
R ³ = propargyl	1.7 ± 0.3
R ⁴ = propargyl	3.7 ± 0.5
R ⁶ = propargyl	2.0 ± 0.9
R ⁷ = propargyl	9.3 ± 3.4
R ⁸ = propargyl	66 ± 15
R ⁹ = propargyl	19 ± 7.6

Figure S1. Structures and A549 cell inhibition of propargylglycine-substituted CbA analogues from amino acid scanning for probe development.

HPLC Chromatograms of Purified CbA Derivatives

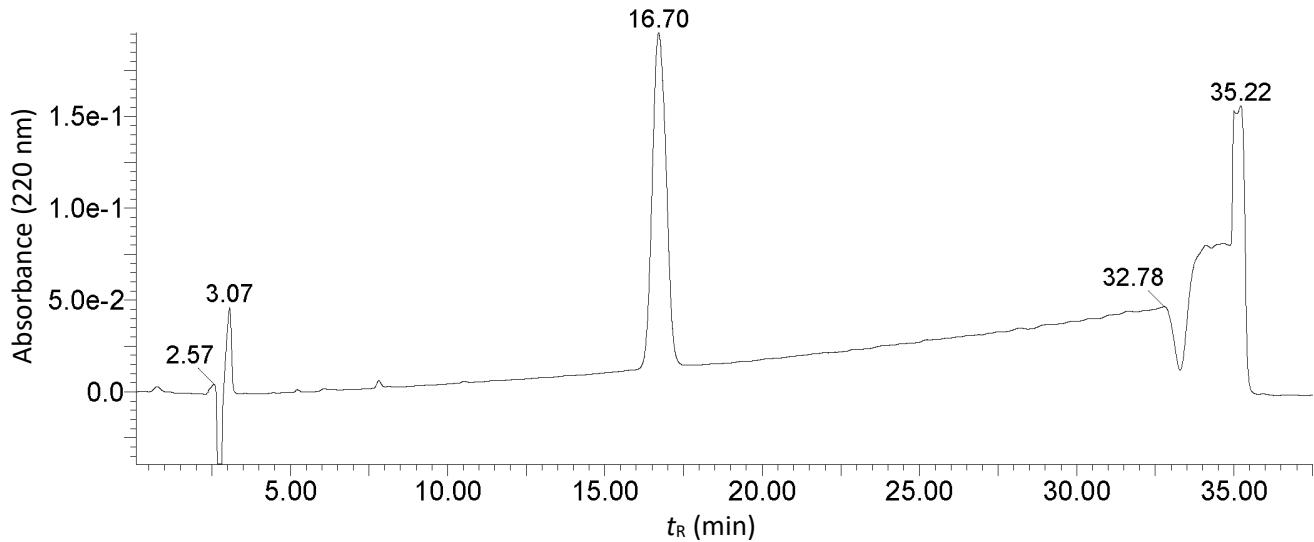


Figure S2. HPLC chromatogram for [MePra3]-CbA ([MePra3]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

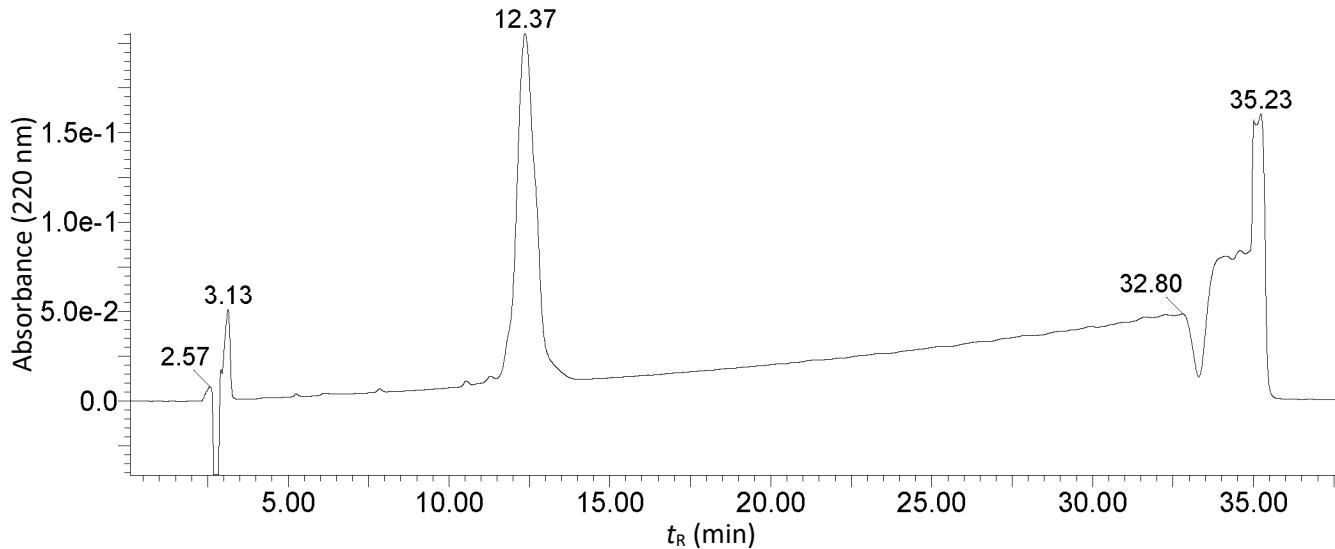


Figure S3. HPLC chromatogram for [MePra4]-CbA ([MePra4]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

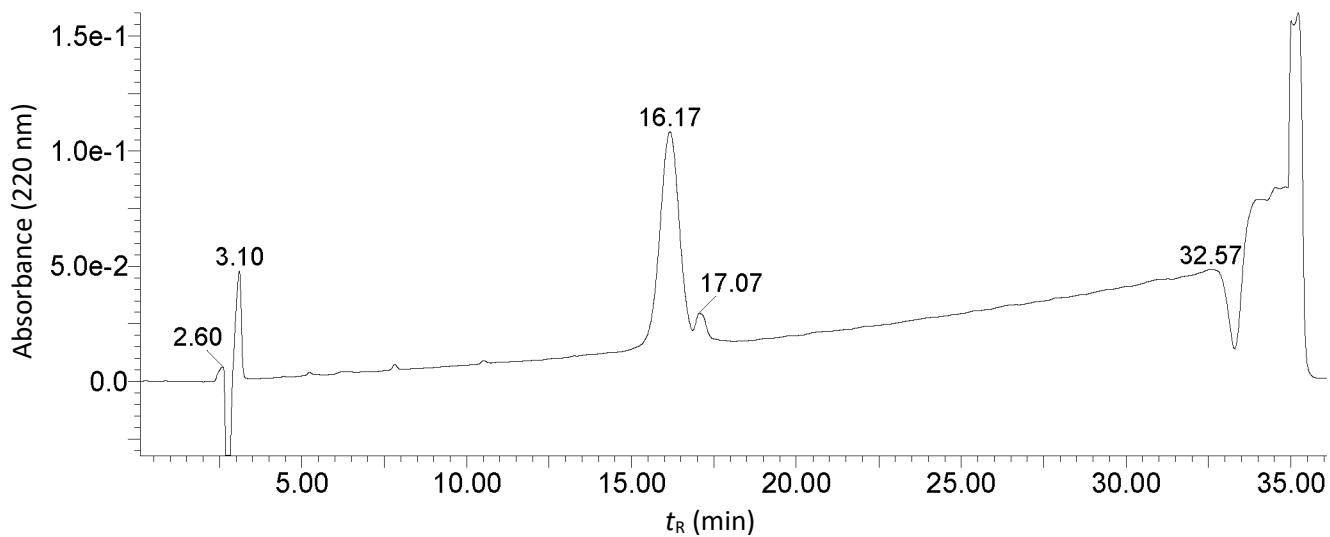


Figure S4. HPLC chromatogram for [MePra6]-CbA ([MePra6]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

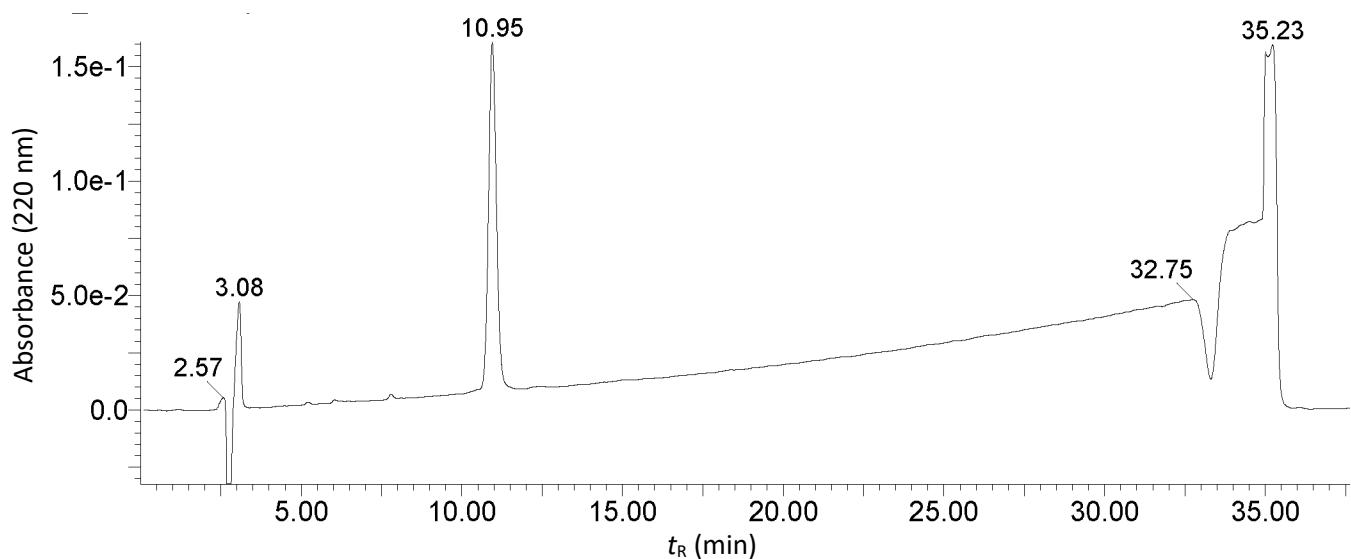


Figure S5. HPLC chromatogram for [MePra7]-CbA ([MePra7]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

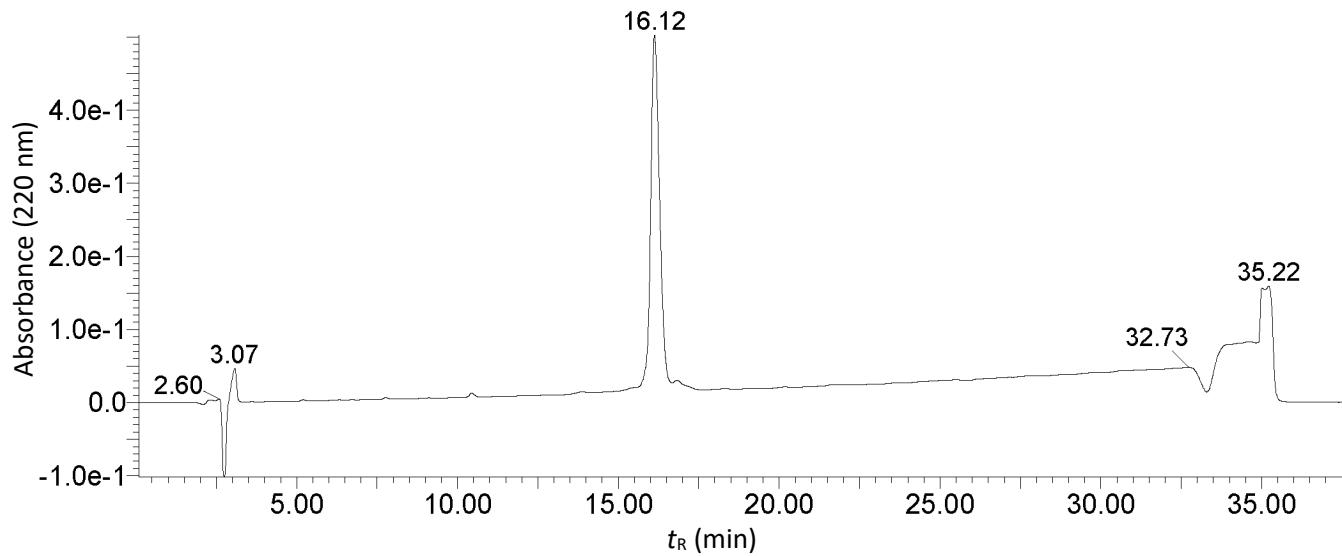


Figure S6. HPLC chromatogram for [Pra8]-CbA ([Pra8]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

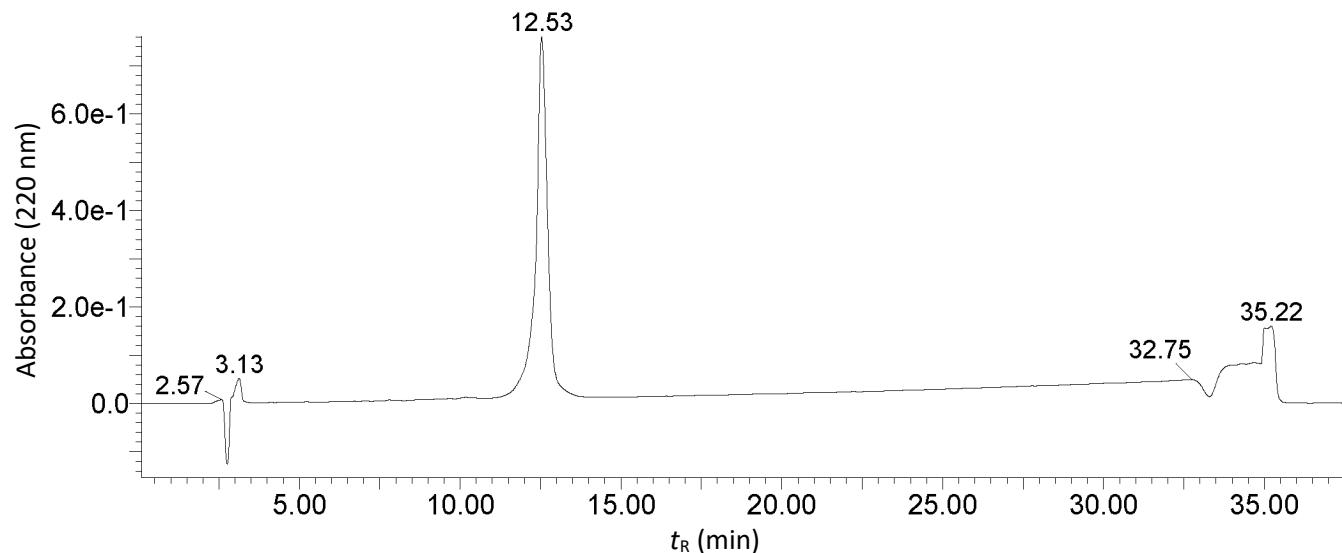


Figure S7. HPLC chromatogram for [MePra9]-CbA ([MePra9]-S12). HPLC conditions: linear gradient of 50-80% CH₃CN containing 0.1% TFA over 30 min at a flow rate of 1 mL/min.

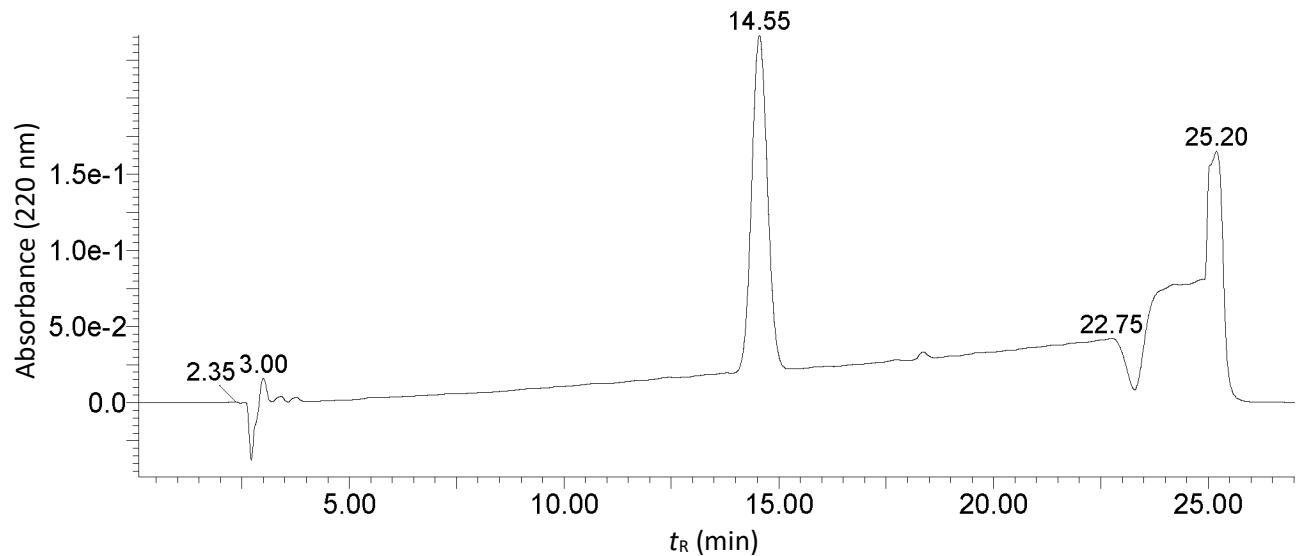


Figure S8. HPLC chromatogram for [Tdf10]-CbA ([Tdf10]-S12). HPLC conditions: linear gradient of 60-80% CH₃CN containing 0.1% TFA over 20 min at a flow rate of 1 mL/min.

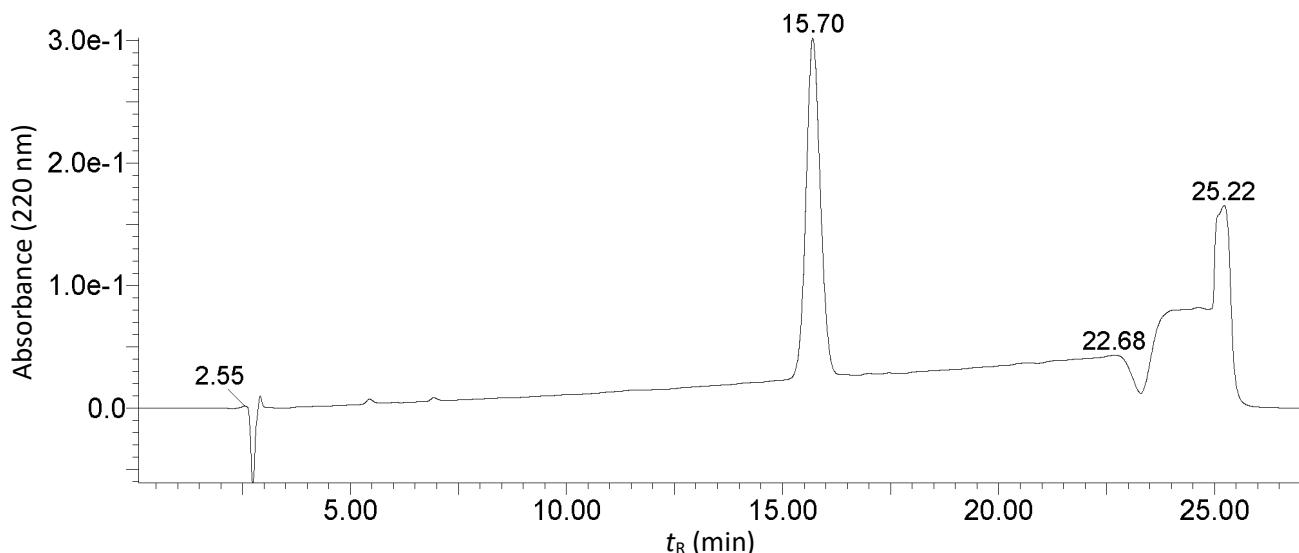


Figure S9. HPLC chromatogram for [MePra3,Tdf10]-CbA ([MePra3,Tdf10]-S12). HPLC conditions: linear gradient of 60-80% CH₃CN containing 0.1% TFA over 20 min at a flow rate of 1 mL/min.

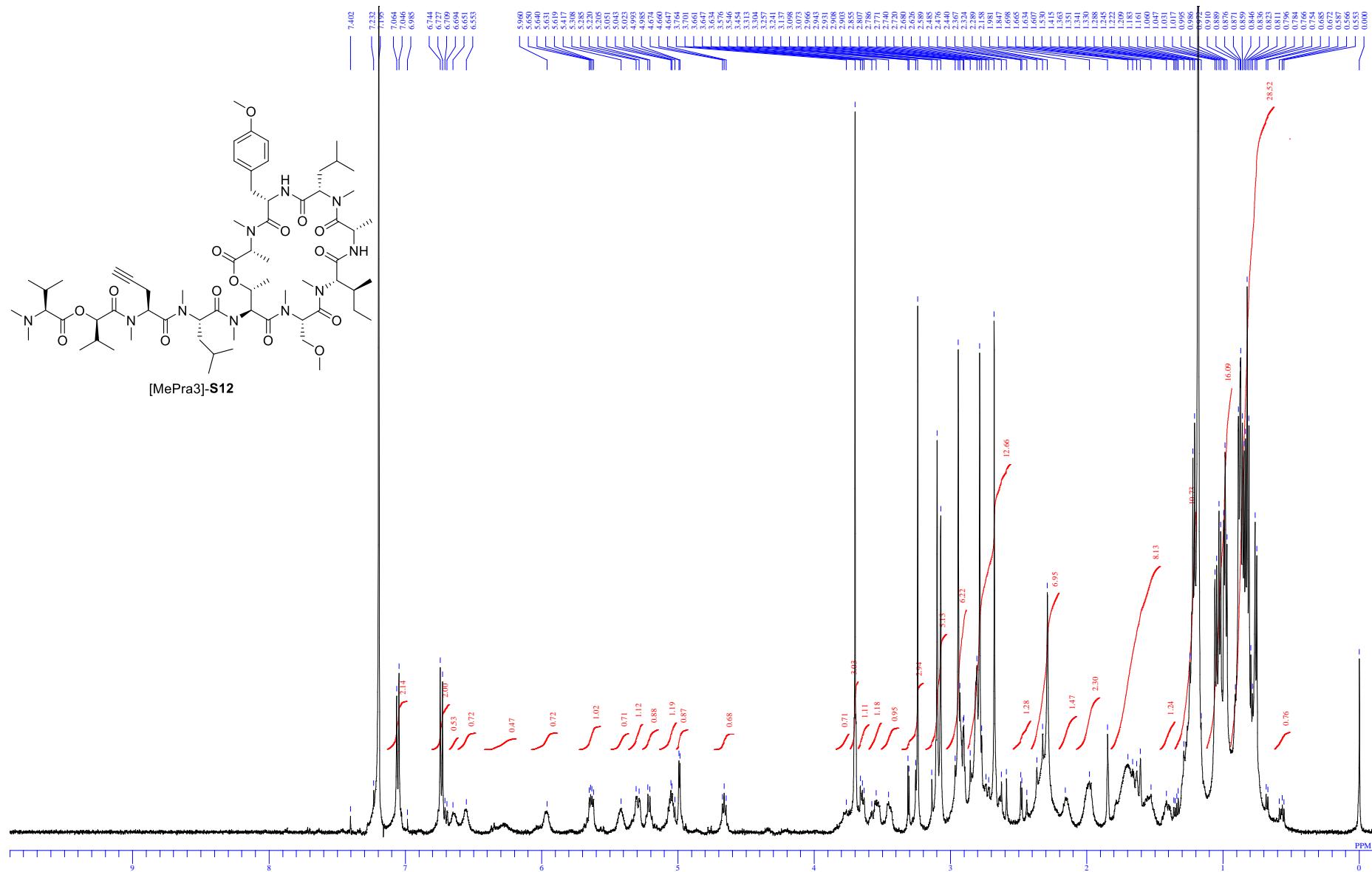


Figure S10. ^1H NMR spectrum for **[MePra3]-S12** at 500 MHz in CDCl_3 .

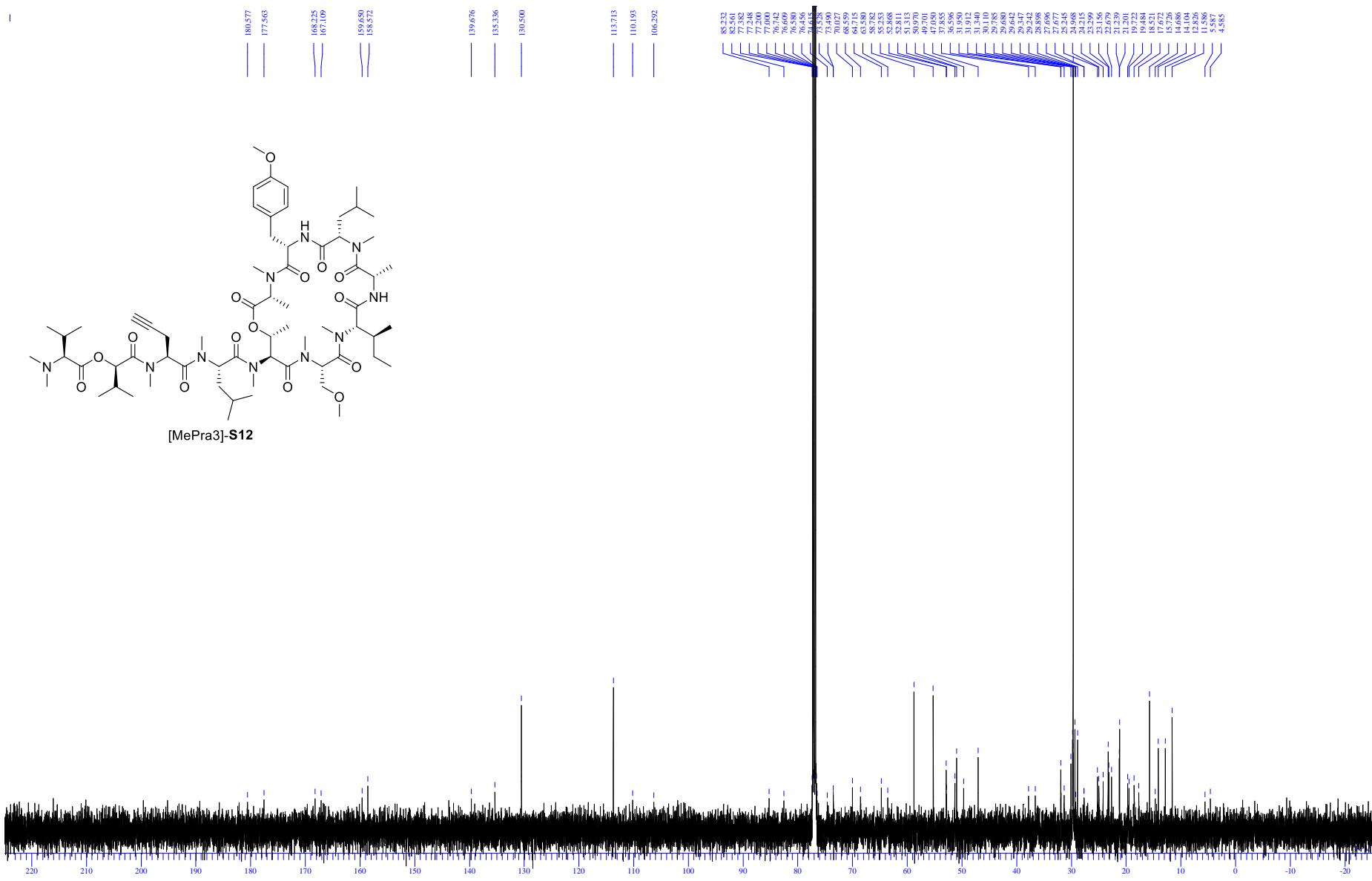


Figure S11. ¹³C NMR spectrum for **[MePra3]-S12** at 125 MHz in CDCl₃.

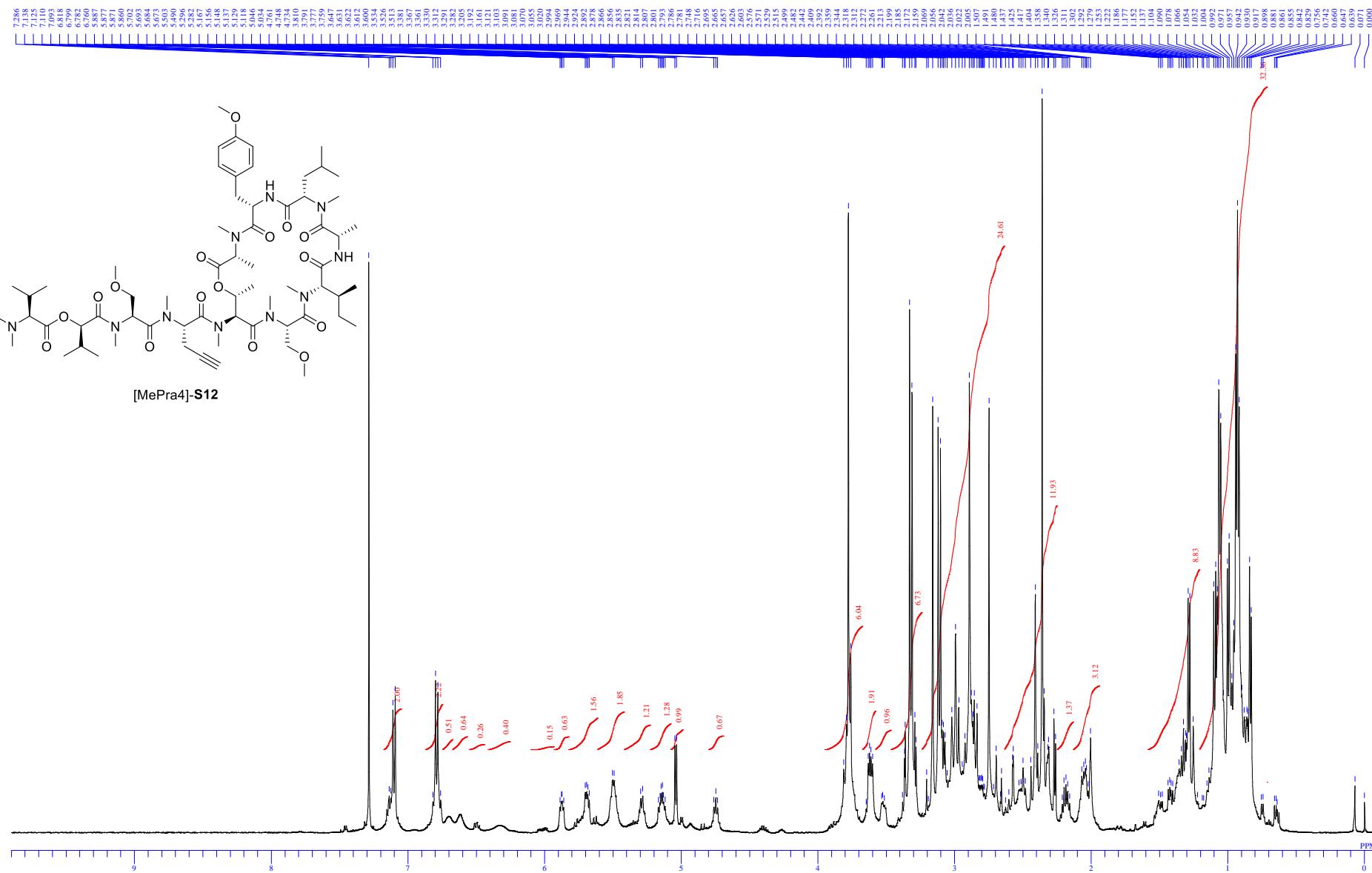


Figure S12. ¹H NMR spectrum for [MePra4]-S12 at 500 MHz in CDCl₃.

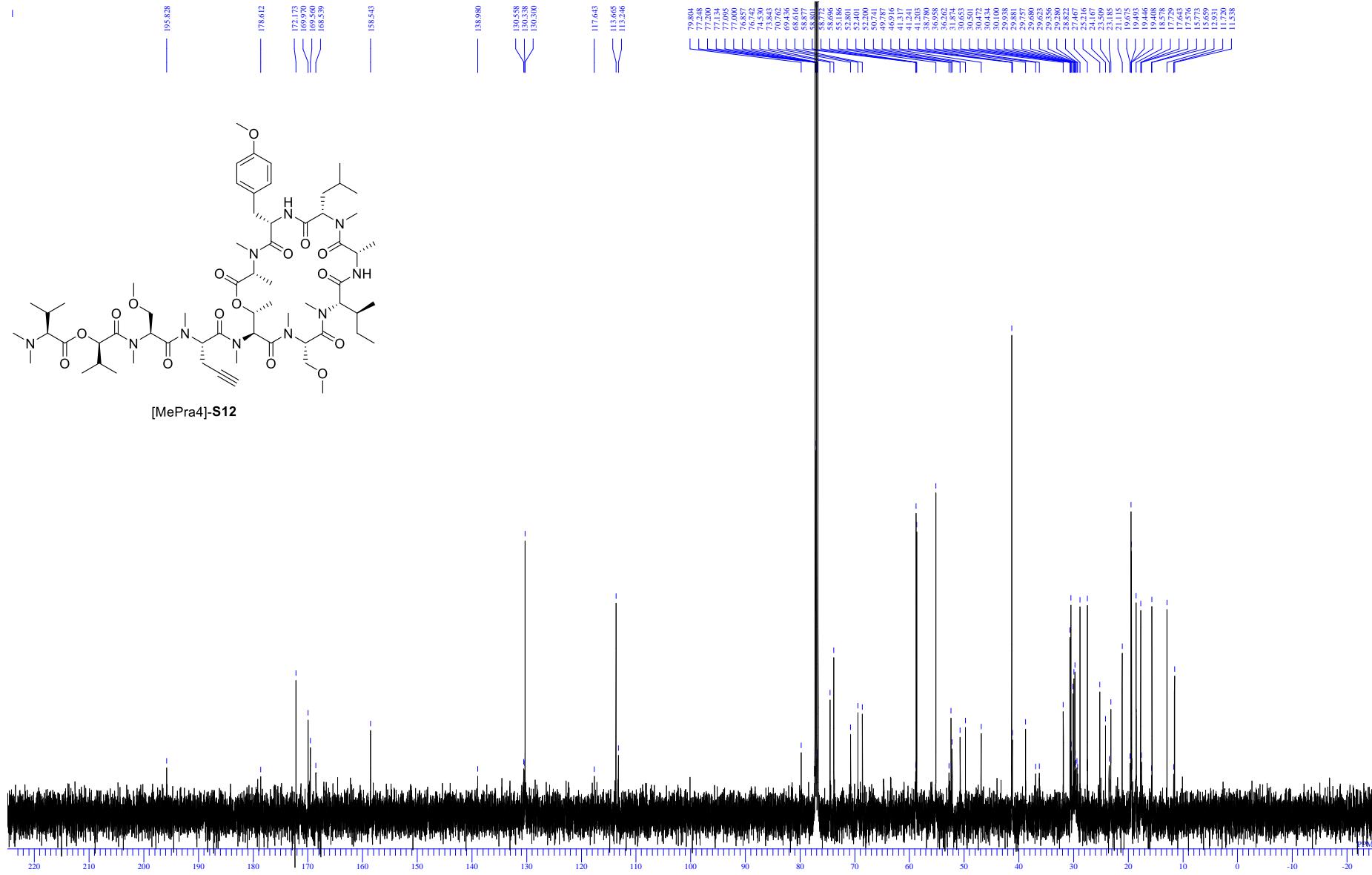


Figure S13. ¹³C NMR spectrum for [MePra4]-S12 at 125 MHz in CDCl₃.

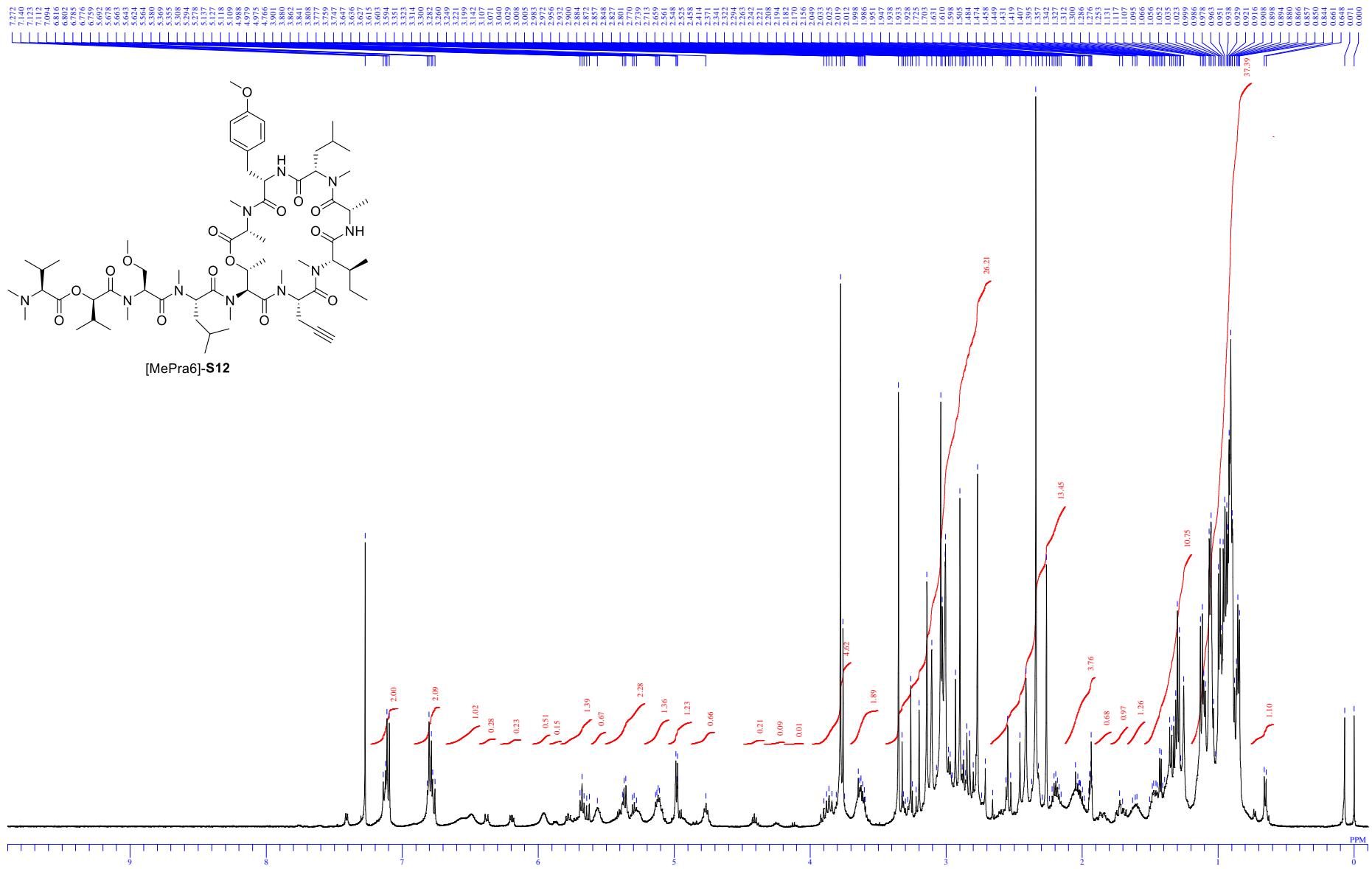


Figure S14. ^1H NMR spectrum for [MePra₆]-S12 at 500 MHz in CDCl_3 .

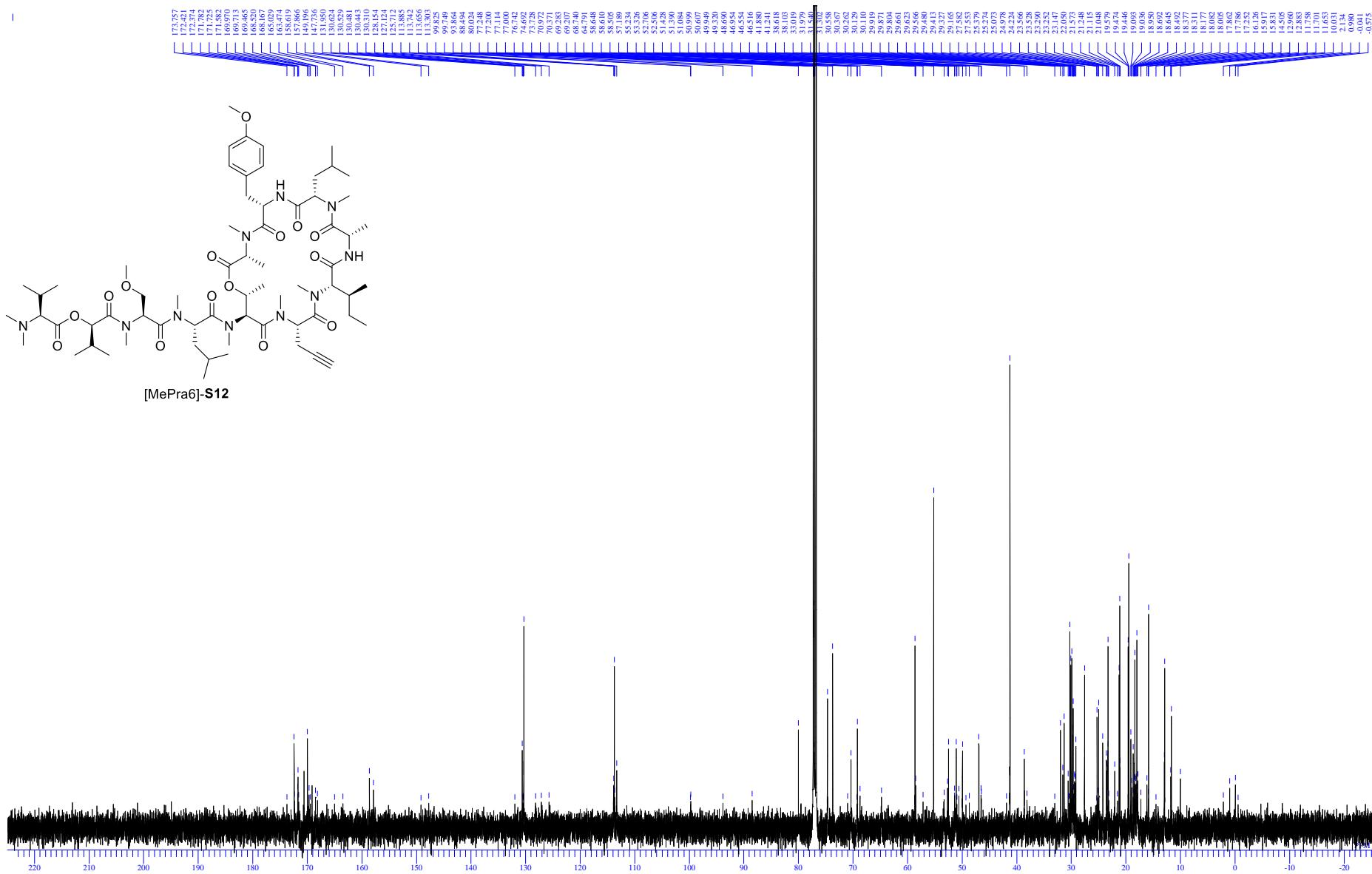


Figure S15. ^{13}C NMR spectrum for [MePra6]-S12 at 125 MHz in CDCl_3 .

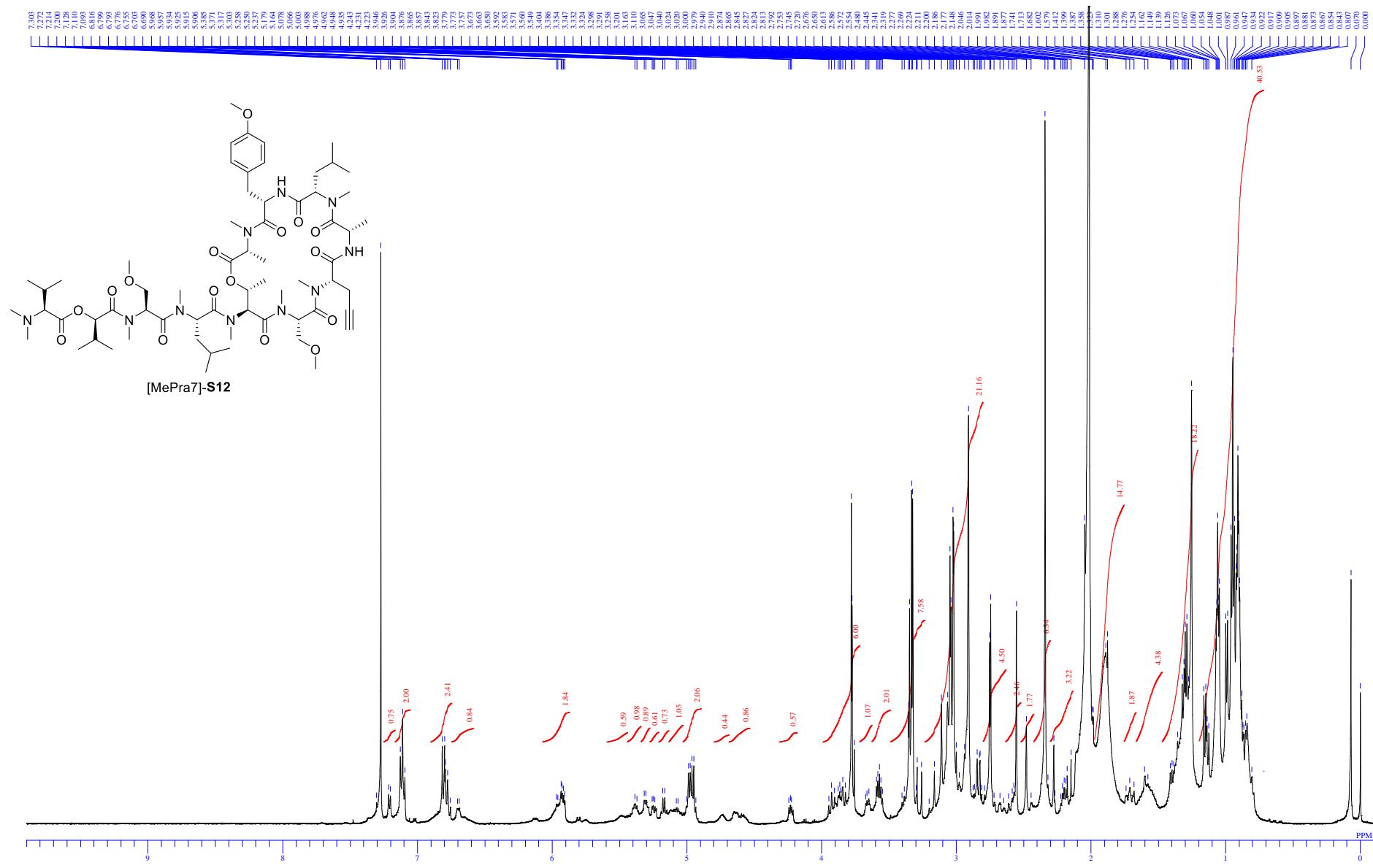


Figure S16. ¹H NMR spectrum for [MePra7]-S12 at 500 MHz in CDCl₃.

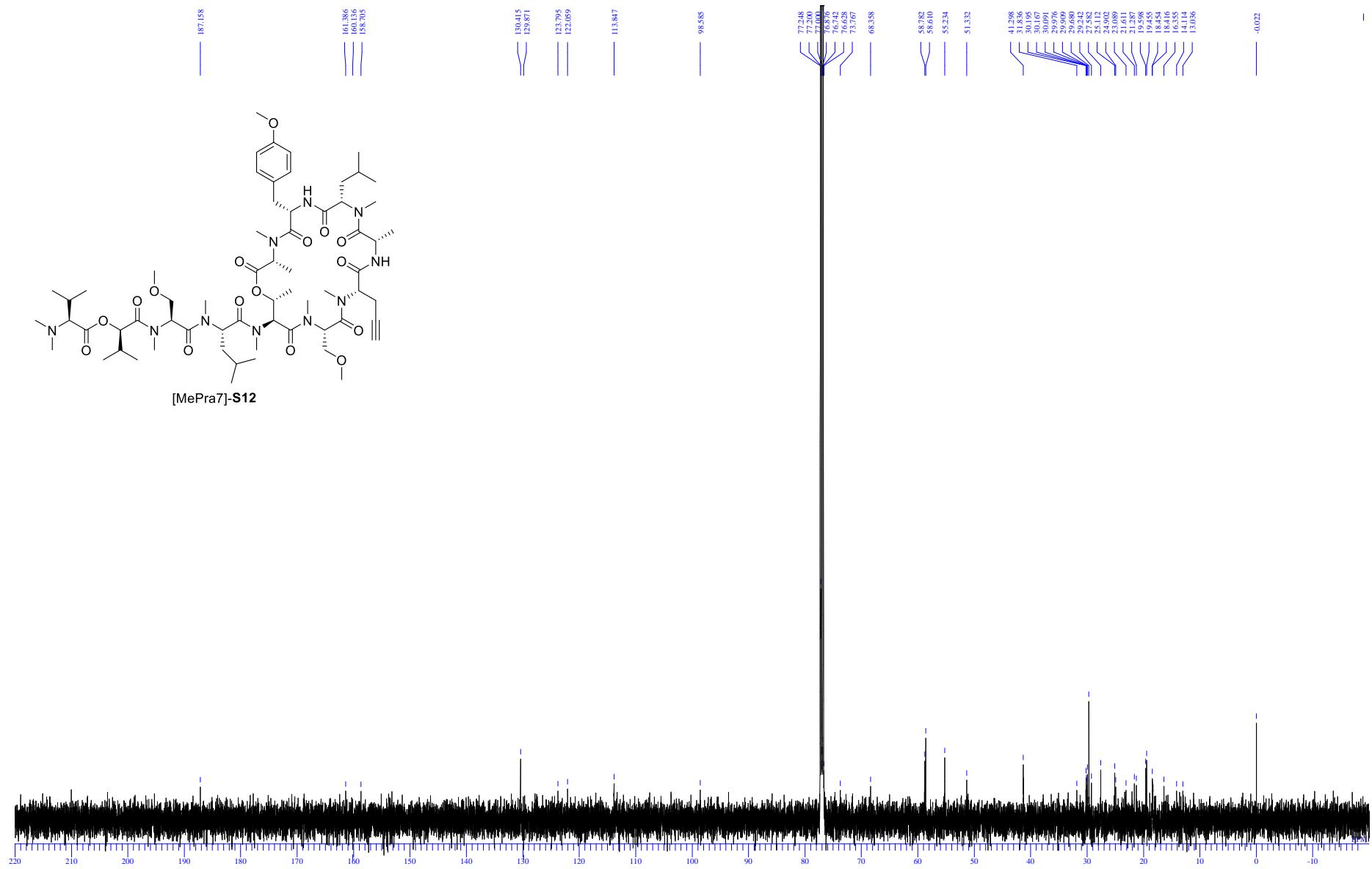


Figure S17. ^{13}C NMR spectrum for [MePra7]-S12 at 125 MHz in CDCl_3 .

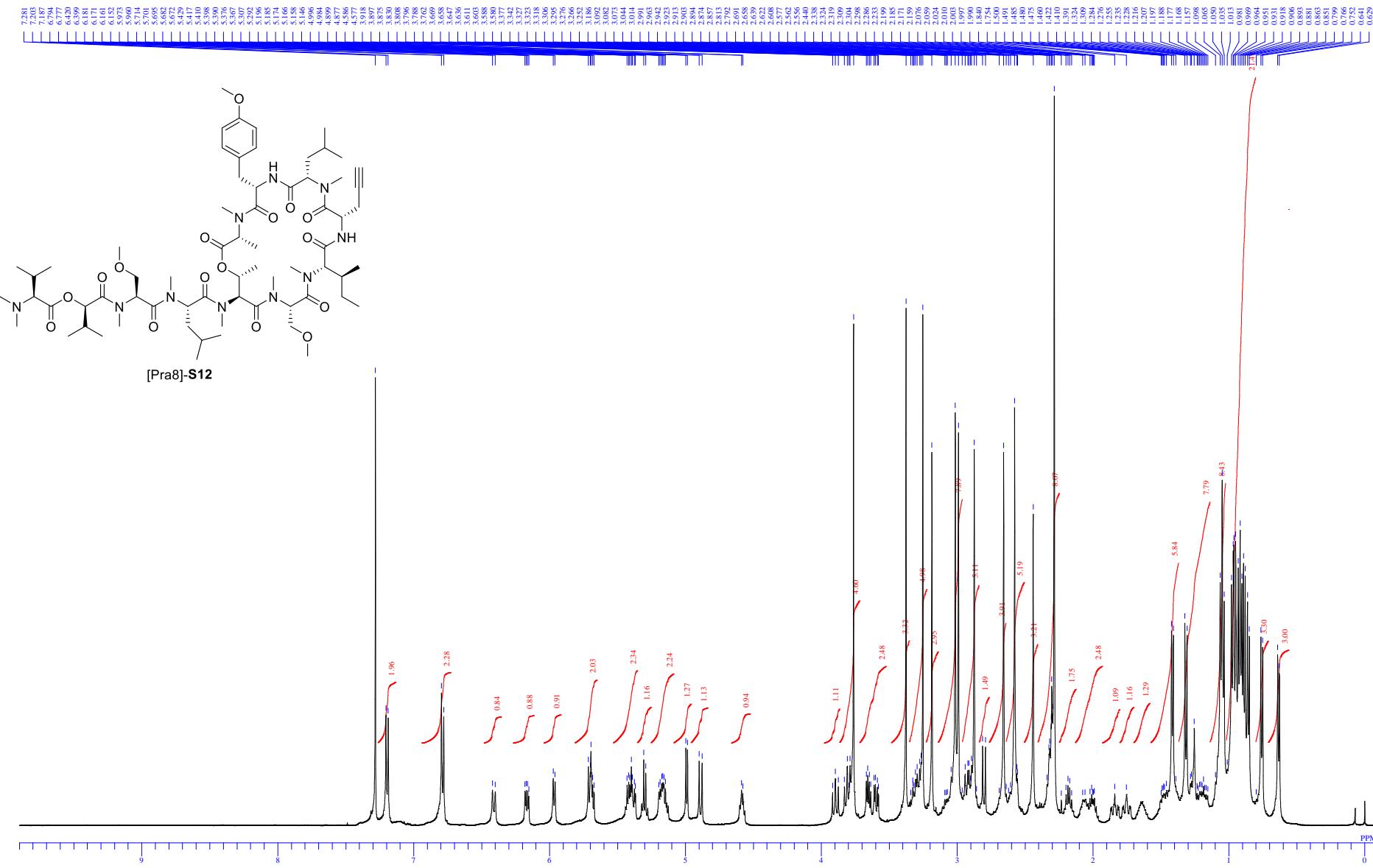


Figure S18. ^1H NMR spectrum for [Pra8]-S12 at 500 MHz in CDCl_3 .

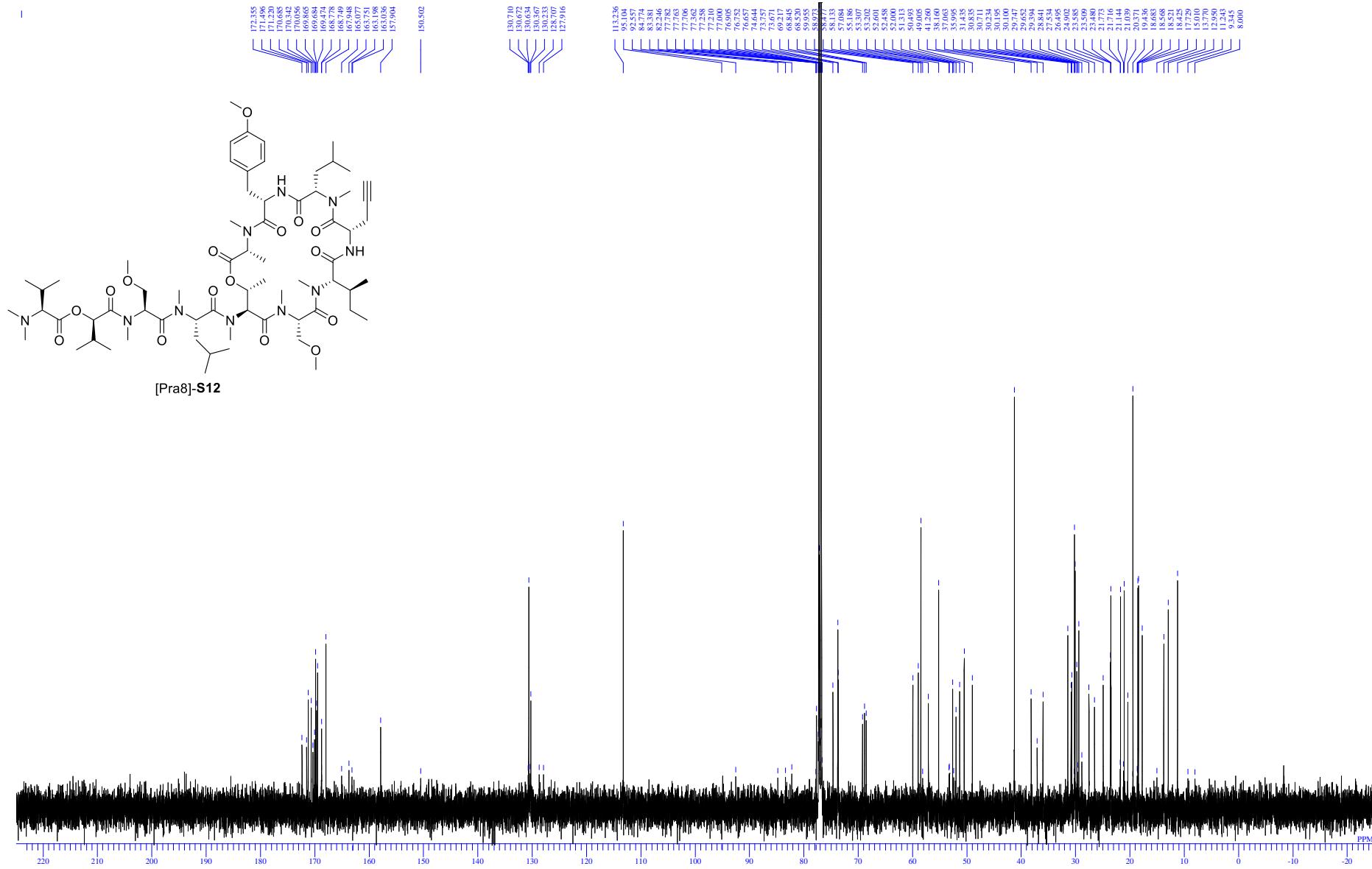


Figure S19. ^{13}C NMR spectrum for [Pra8]-S12 at 125 MHz in CDCl_3 .

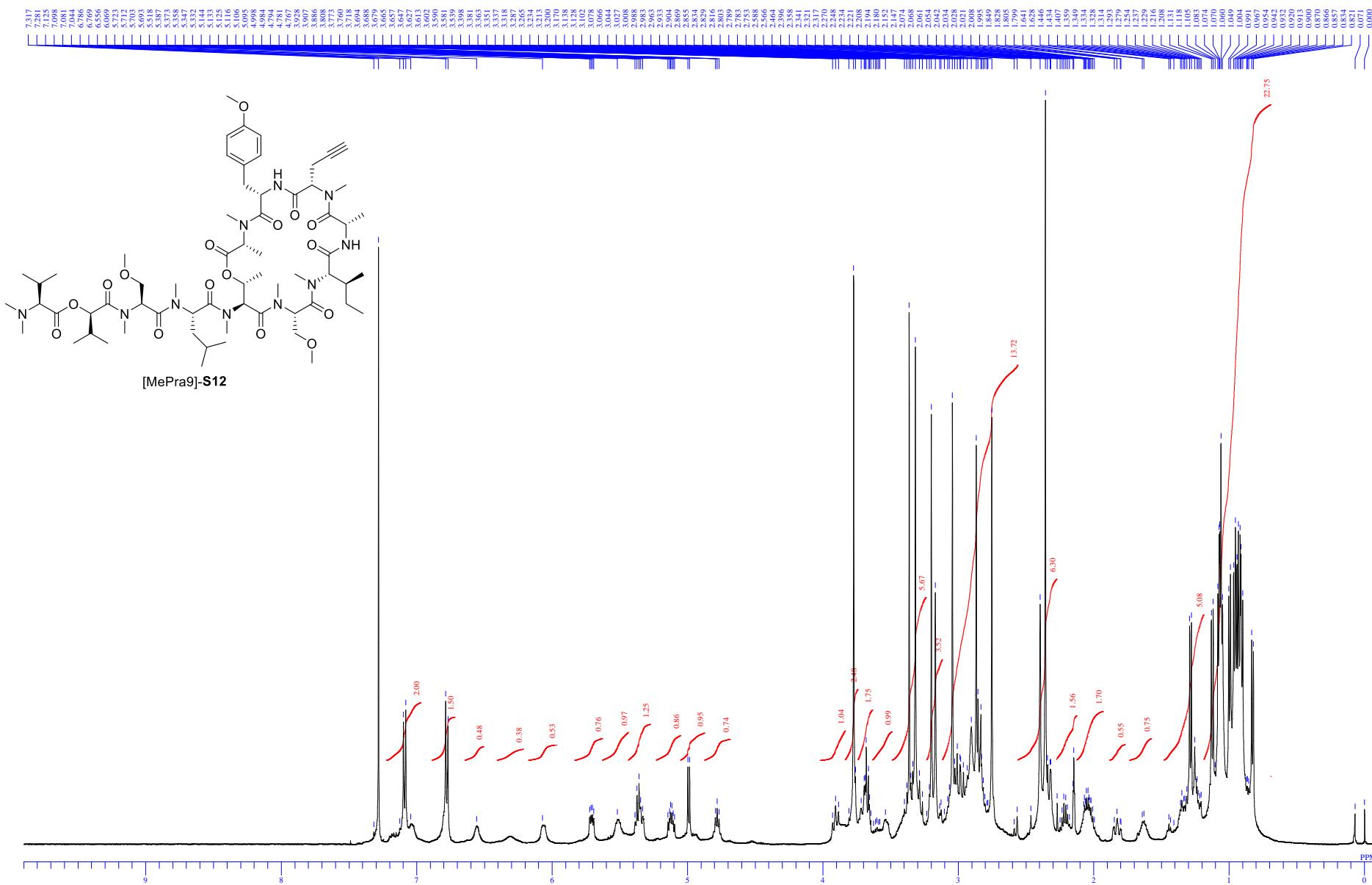


Figure S20. ^1H NMR spectrum for [MePra9]-S12 at 500 MHz in CDCl_3 .

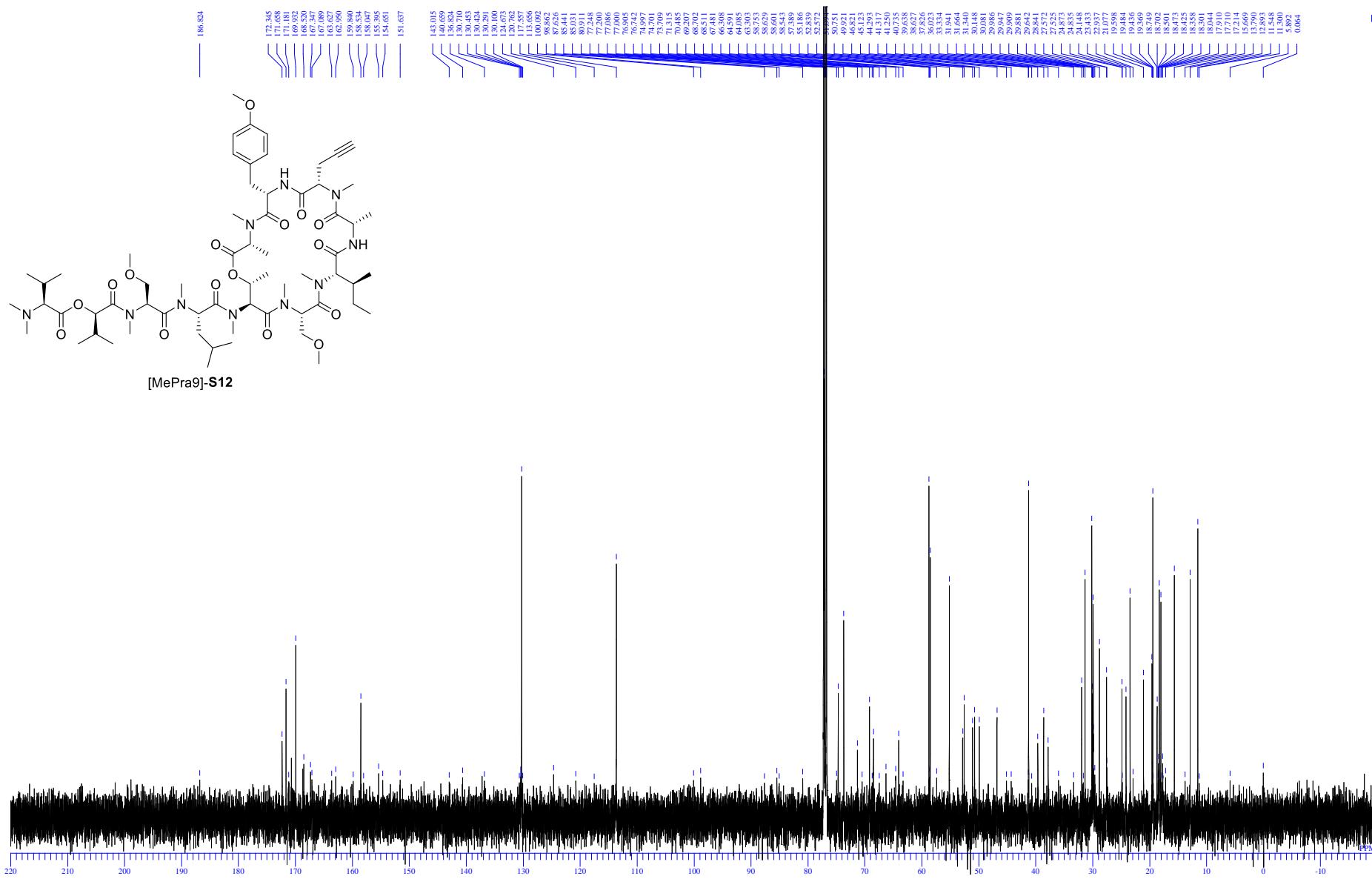
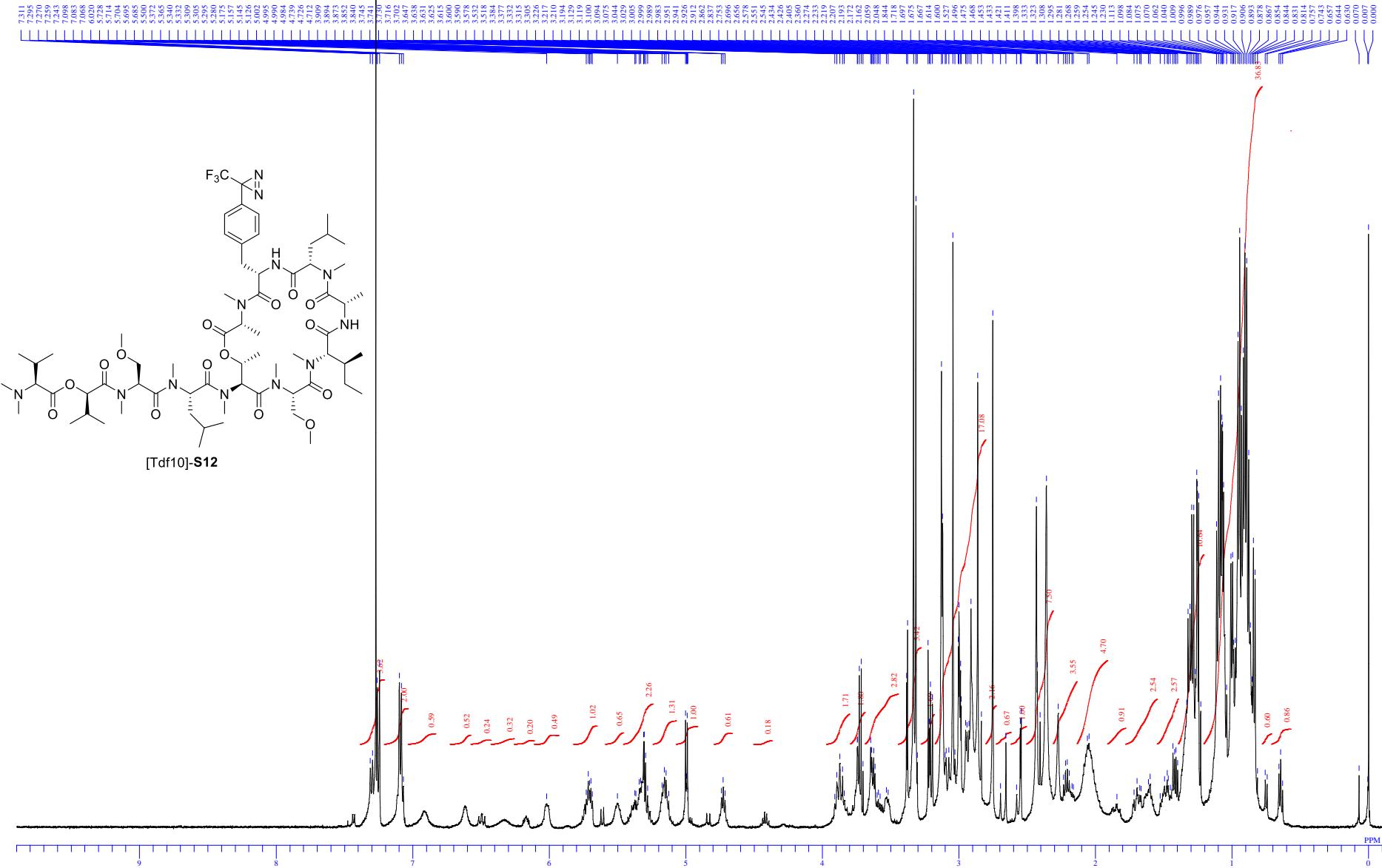


Figure S21. ^{13}C NMR spectrum for [MePra9]-S12 at 125 MHz in CDCl_3 .



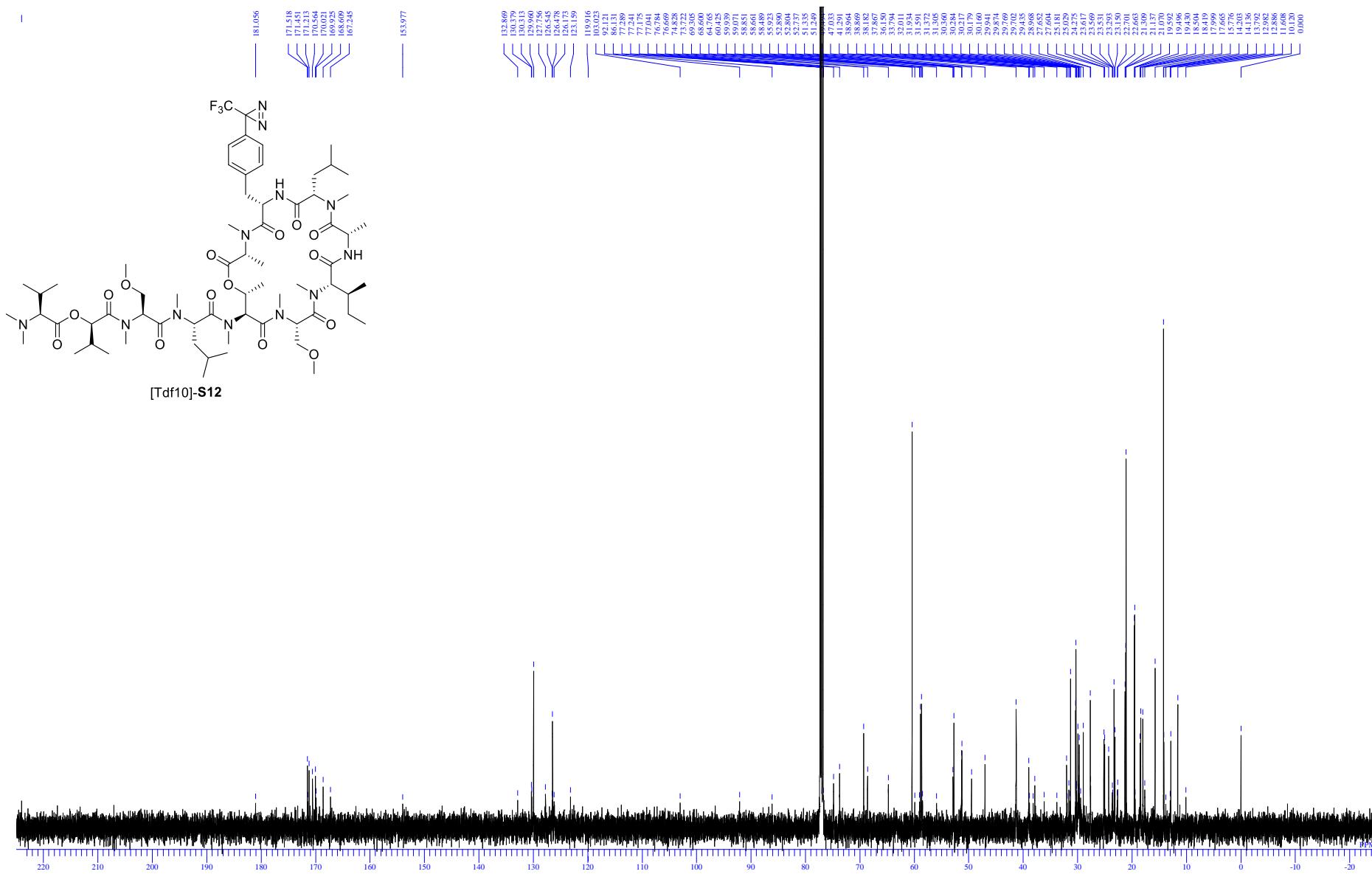


Figure S23. ^{13}C NMR spectrum for [Tdf10]-S12 at 125 MHz in CDCl_3 .

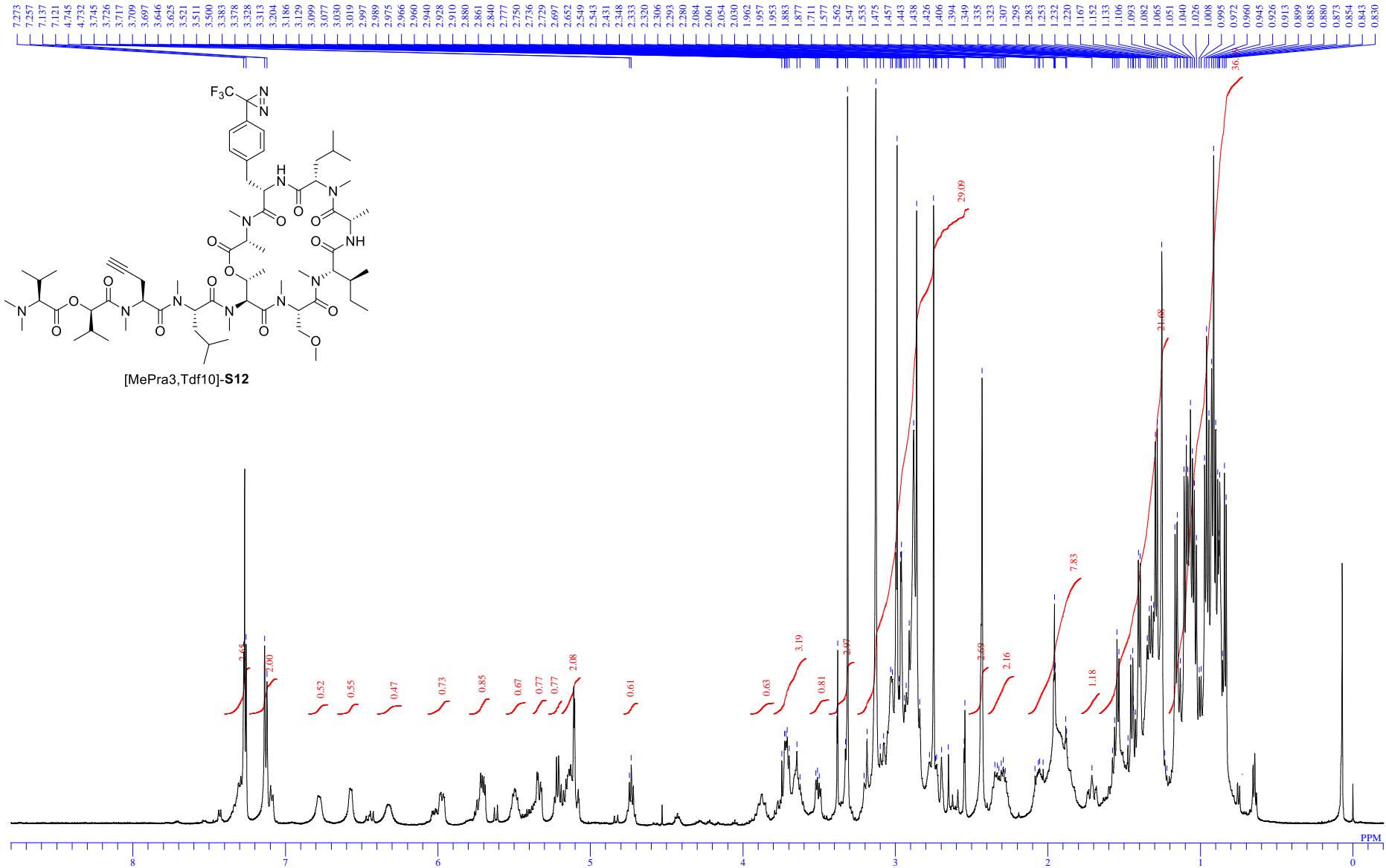


Figure S24. ¹H NMR spectrum for [MePra3,Tdf10]-S12 at 500 MHz in CDCl₃.

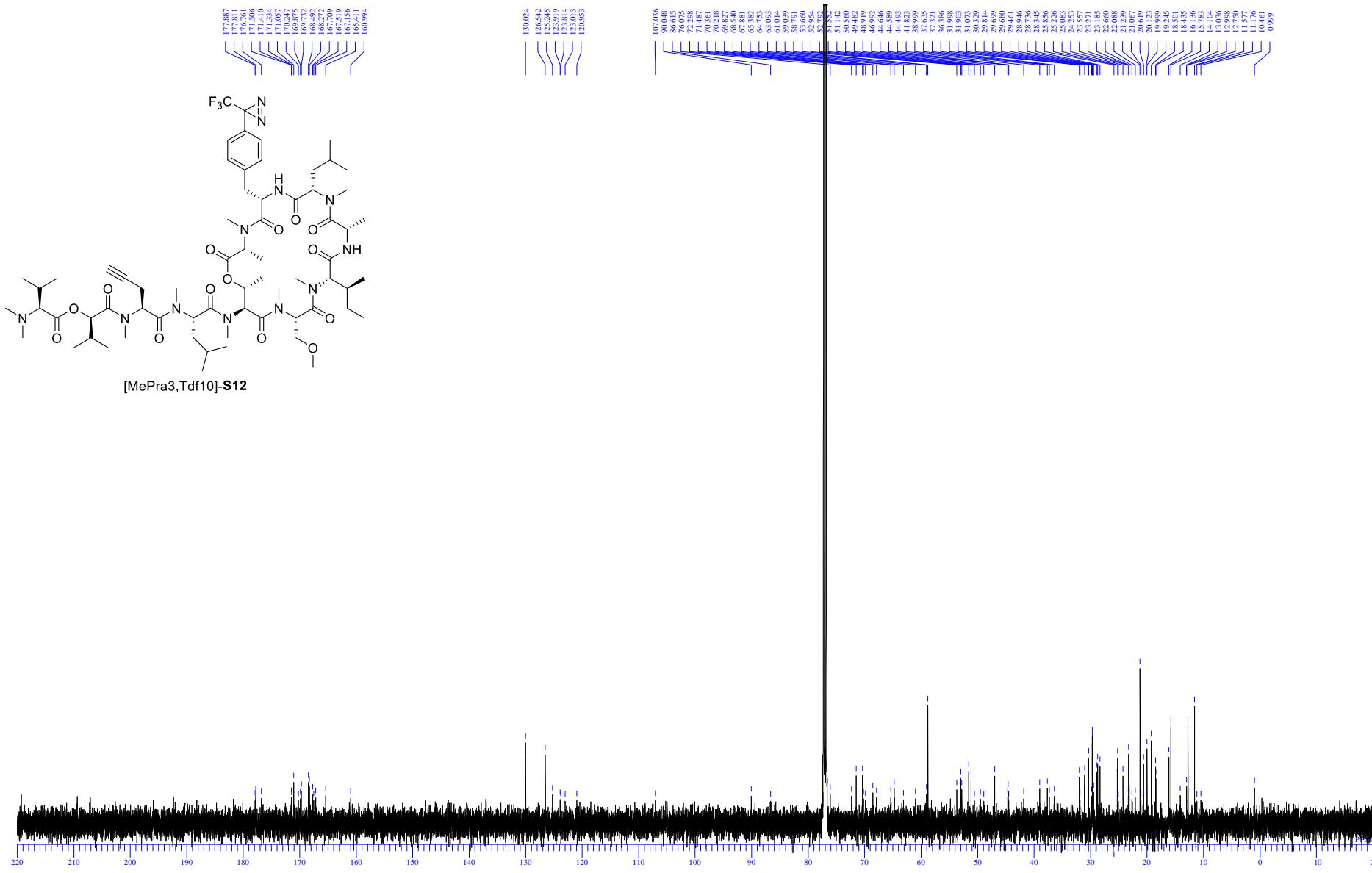


Figure S25. ^{13}C NMR spectrum for $[\text{MePra3},\text{Tdf10}]\text{-S12}$ at 125 MHz in CDCl_3 .

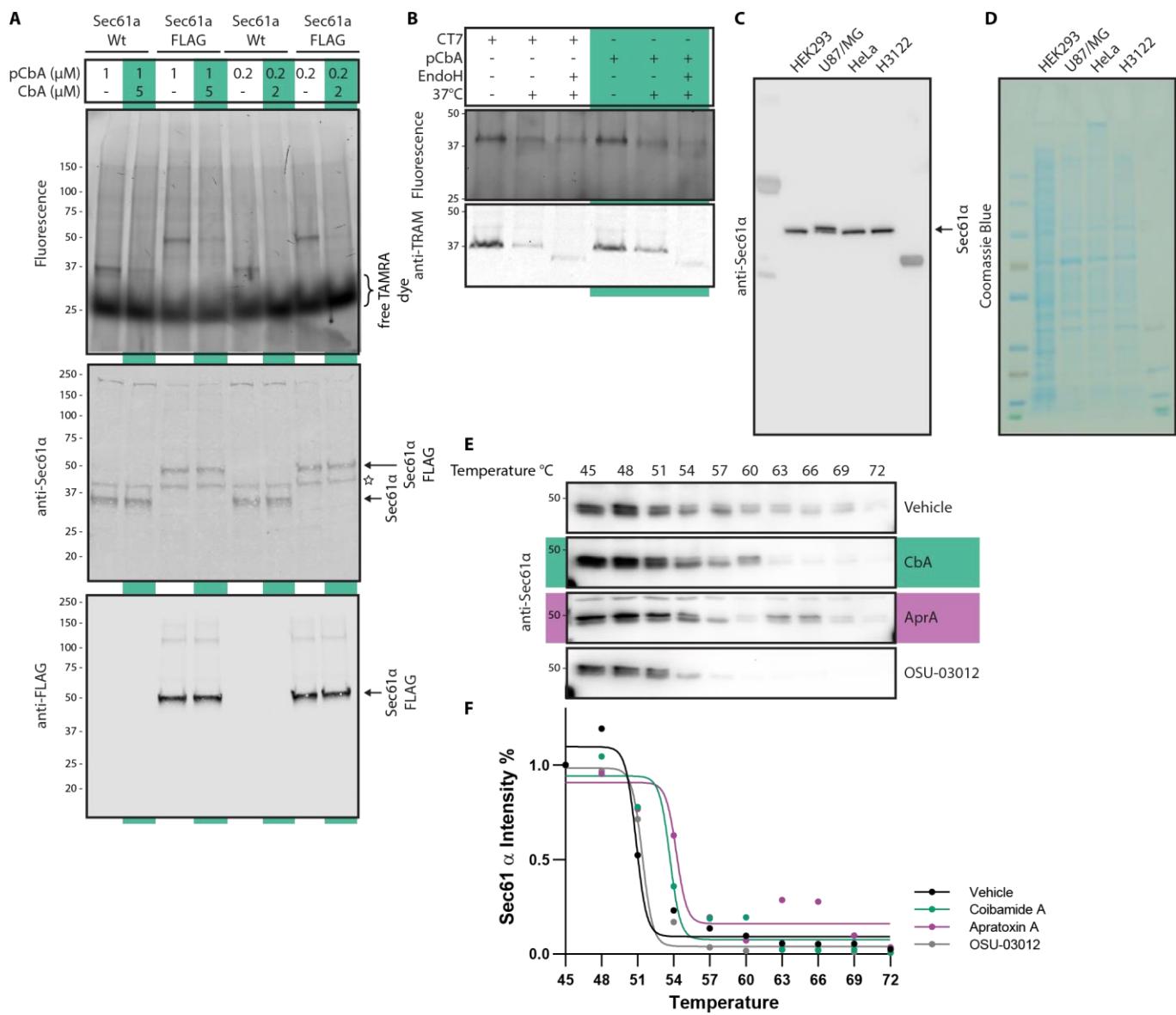


Figure S26. Photo-CbA crosslinking and thermal stabilization assays.

(A) Same as Fig 2A, but uncropped gels including an additional concentration point. (B) Photocrosslinked adducts prepared as Fig 2B using photo-CbA or CT7, incubated in the presence or absence of Endoglycosidase H, then detected by TAMRA fluorescence or anti-TRAM immunoblot (C) Immunoblot analysis of Sec61 α expression in human embryonic kidney HEK293 (lane 2), U87-MG glioblastoma (lane 3), HeLa cervical cancer (lane 4) and H3122 lung cancer cell lysates (lane 5). (D) Identical gel to C stained with Coomassie Blue dye. Protein molecular weight markers are 70 kDa and 35 kDa in lane 1, and 40 kDa in lane 6. (E) Immunoblot analysis of soluble fraction of U87-MG cells

following treatment with 1 μ M coibamide A, 1 μ M apratoxin A, 3 μ M OSU-03012 or vehicle (0.1% DMSO) for 1 h prior to heat treatment. Cells were distributed into PCR tubes and subjected to a designated temperature (45°C to 72°C) for 3 min, incubated at room temperature for 3 min and subjected to snap-freezing. Cell lysates were cleared by centrifugation and the soluble fraction processed for Western blot analysis with anti-Sec61 α . (F) Quantification of data shown in E. Apparent melting curves for Sec61 α were calculated by densitometry where Sec61 α intensity at each temperature was compared to signal at 45°C for each treatment. The figure is a representative of three independent experiments.

Table S1. US National Cancer Institute 60 cancer cell line (NCI60) panel Matrix COMPARE analysis.

Row Label	COIBAMIDE A	APRATOXIN A	IPOMOEASSIN F	MYCOLACTONE
	NSC:S742997 Endpt:GI50 AVGDATA hiConc:-5.0	NSC:S722167 Endpt:GI50 AVGDATA hiConc:-5.0	NSC:S791000 Endpt:GI50 hiConc:-4.0	NSC:S709758 Endpt:GI50 hiConc:-4.3
NSC:S742997 Endpt:GI50 AVGDATA hiConc:-5.0 COIBAMIDE A	correlation: 1.0 count cell lines: 59 seed stdDev: 0.695 target stdDev: 0.695	correlation: 0.374 count cell lines: 57 seed stdDev: 0.702 target stdDev: 0.951	correlation: 0.562 count cell lines: 58 seed stdDev: 0.685 target stdDev: 0.711	correlation: 0.591 count cell lines: 54 seed stdDev: 0.696 target stdDev: 0.854
NSC:S722167 Endpt:GI50 AVGDATA hiConc:-5.0 APRATOXIN A	correlation: 0.374 count cell lines: 57 seed stdDev: 0.951 target stdDev: 0.702	correlation: 1.0 count cell lines: 57 seed stdDev: 0.951 target stdDev: 0.951	correlation: 0.383 count cell lines: 56 seed stdDev: 0.955 target stdDev: 0.717	correlation: 0.436 count cell lines: 52 seed stdDev: 0.964 target stdDev: 0.856
NSC:S791000 Endpt:GI50 hiConc:-4.0 IPOMOEASSIN F	correlation: 0.562 count cell lines: 58 seed stdDev: 0.711 target stdDev: 0.685	correlation: 0.383 count cell lines: 56 seed stdDev: 0.717 target stdDev: 0.955	correlation: 1.0 count cell lines: 59 seed stdDev: 0.708 target stdDev: 0.708	correlation: 0.435 count cell lines: 54 seed stdDev: 0.731 target stdDev: 0.854
NSC:S709758 Endpt:GI50 hiConc:-4.3 MYCOLACTONE	correlation: 0.591 count cell lines: 54 seed stdDev: 0.854 target stdDev: 0.696	correlation: 0.436 count cell lines: 52 seed stdDev: 0.856 target stdDev: 0.964	correlation: 0.435 count cell lines: 54 seed stdDev: 0.854 target stdDev: 0.731	correlation: 1.0 count cell lines: 54 seed stdDev: 0.854 target stdDev: 0.854

Table S2. Comparison of published NCI60 panel data for Coibamide A,¹ Apratoxin A² and Ipomeoassin F³

NCI60 Cell Line	Coibamide A	GI50 [M]		
		Apratoxin A	Ipomeoassin F	nd = NO DATA
HCC-2998	1.00E-09	3.65E-09	1.18E-06	
HT29	1.00E-09	1.04E-09	1.00E-08	
SK-MEL-5	1.00E-09	2.31E-09	1.00E-08	
MCF7	1.00E-09	6.57E-09	1.00E-08	
T-47D	1.00E-09	3.63E-09	1.00E-08	
NCI-H460	1.44E-09	2.77E-09	1.00E-08	
SF-539	1.44E-09	5.03E-10	1.00E-08	
SF-295	1.50E-09	1.26E-09	1.00E-08	
SF-268	1.53E-09	5.08E-08	1.15E-07	
HL-60 (TB)	1.66E-09	8.51E-10	4.90E-08	
COLO 205	1.83E-09	2.71E-09	1.00E-08	
HOP-92	1.93E-09	3.09E-09	1.00E-08	
SK-OV-3	1.98E-09	5.27E-09	3.84E-08	
LOX IMVI	2.13E-09	2.30E-09	1.00E-08	
M14	2.20E-09	2.09E-09	1.00E-08	
HCT-116	2.81E-09	9.16E-10	1.00E-08	
SR	2.94E-09	1.50E-09	1.44E-08	
MDA-MB-231/ATCC	3.59E-09	1.46E-09	1.00E-08	
HS 578T	3.71E-09	nd	1.00E-08	
RXF 393	3.90E-09	7.12E-09	1.00E-08	
RPMI-8226	3.93E-09	1.28E-09	1.00E-08	
IGROV1	4.10E-09	7.42E-10	2.67E-08	
U251	4.11E-09	1.56E-09	1.00E-08	
K-562	4.41E-09	1.48E-08	1.00E-08	
SNB-75	4.52E-09	1.96E-09	1.00E-08	
SW-620	5.29E-09	3.16E-09	1.00E-08	
CCRF-CEM	5.34E-09	nd	7.41E-07	
MDA-MB-435	5.95E-09	3.35E-09	1.00E-08	
SNB-19	6.31E-09	7.00E-09	1.29E-07	
HOP-62	6.96E-09	7.68E-09	1.00E-08	
PC-3	7.13E-09	4.96E-09	1.00E-08	
SK-MEL-28	8.65E-09	5.49E-09	1.75E-08	
NCI-H226	1.09E-08	1.31E-09	2.76E-07	
UO-31	1.48E-08	2.95E-09	1.00E-08	
OVCAR8	1.60E-08	7.26E-09	1.17E-07	
786-0	1.64E-08	4.52E-09	3.01E-08	
A549/ATCC	1.85E-08	5.38E-09	1.00E-08	
A498	1.86E-08	nd	3.15E-07	
NCI-H522	1.94E-08	6.03E-09	2.79E-08	
DU-145	2.13E-08	1.03E-08	3.19E-08	
UACC-257	2.19E-08	7.15E-09	1.19E-08	
UACC-62	2.63E-08	3.28E-09	1.53E-08	
MALME-3M	2.64E-08	4.77E-09	5.78E-08	
KM12	3.01E-08	3.52E-09	1.00E-08	
NCI-H322M	3.39E-08	3.99E-09	3.91E-07	
ACHN	4.14E-08	2.69E-09	nd	
SN12C	4.24E-08	3.94E-09	1.00E-08	
OVCAR5	4.80E-08	1.00E-07	1.23E-06	
EKVV	5.79E-08	7.56E-09	1.00E-08	
OVCAR3	7.40E-08	1.69E-08	2.83E-08	
HCT-15	7.73E-08	4.29E-09	2.98E-08	
MOLT-4	1.12E-07	4.80E-08	3.42E-06	
SK-MEL-2	1.45E-07	1.00E-07	4.45E-07	
NCI/ADR-RES	2.87E-07	1.53E-08	2.97E-07	
NCI-H23	3.03E-07	8.23E-09	2.13E-07	
CAKI-1	4.06E-07	4.05E-09	nd	
TK-10	4.24E-07	2.63E-09	1.52E-08	
BT-549	1.03E-06	nd	1.79E-08	
OVCAR4	1.59E-06	1.00E-07	1.58E-06	

References:

1. Medina, R. A.; Goeger, D. E.; Hills, P.; Mooberry, S. L.; Huang, N.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H.; McPhail, K. L. Coibamide A, a Potent Antiproliferative Cyclic Depsipeptide from the Panamanian Marine Cyanobacterium *Leptolyngbya* Sp. *J. Am. Chem. Soc.* **2008**, *130*, 6324–6325.
2. Luesch, H.; Chanda, S. K.; Raya, R. M.; DeJesus, P. D.; Orth, A. P.; Walker, J. R.; Izpisúa-Belmonte, J. C.; Schultz, P. G. A Functional Genomics Approach to the Mode of Action of Apratoxin A. *Nat. Chem. Biol.* **2006**, *2*, 158–167.
3. Zong, G.; Whisenhunt, L.; Hu, Z.; Shi, W. Q. Synergistic Contribution of Tiglate and Cinnamate to Cytotoxicity of Ipomoeassin F. *J. Org. Chem.* **2017**, *82*, 4977–4985.